CHEMICAL COMPOSITION OF ATTRACTIVE BLENDS IN SELECTED *OCIMUM* SPECIES TRADITIONALLY USED TO LURE HONEY BEES

BY

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been presented for a degree in any other University. No part ot this thesis may be reproduced without the prior written permission of author and/or University of Eldoret.

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DEDICATION

This thesis is dedicated to:

My daughter Lilies

It documents scientific evidence in support of a bee luring technology practiced by our forefathers for generations, oblivious of its great potential in transformation of global beekeeping sector.

ABSTRACT

The success of sustainable beekeeping depends on possibility of establishing a bee colony. Low hive occupation rate has been identified as one of the prominent challenges facing beekeeping sector in addition to pest attacks, inadequate skills in hive management and poor apiary conditions. To initiate prompt hive colonization, some beekeepers traditionally use aromatic herbs and shrubs to smoke interior parts of a new hive. However, there is limited scientific evidence supporting the traditional use of aromatic plants as honey bee swarm lures. The aim of this study was to establish the chemical basis of the use of indigenous plants as honey bee lures. Essential oils, fresh and smoldered volatiles of three *Ocimum* species namely; *O. kilimandscharicum, O. kenyense* and *O. lamiifolium* were investigated for their chemical compositions and potential to lure bees. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used to determine chemical composition of volatiles of the *Ocimum* species. Dual choice Y-tube olfactometer experiments were used to determine the attraction of honey bees (*Apis mellifera scutellata*) to choices of essential oil, fresh and smoldered volatiles against negative and positive controls. Swarm luring activity of most attractive *Ocimum* species' volatiles was tested in the field in an 8 by 8 randomized Latin square block experiment. There were notable variations in chemical constituents of the studied *Ocimum* species based on species, agro ecological zone and mode of extraction. Monoterpenoids (49.1-75.1%), benzenoids (28.9-43.9%) and sesquiterpenoids (2.13-37.7%) were the major classes of natural products characterizing respective volatiles of *O. kilimandscharicum, O. kenyense* and *O. lamiifolium* species. (Eucalyptol, estragole and β-pinene), (camphor, linalool and eucalyptol) and (α-phellandrene and β-sabinene) were identified as major chemical constituents of essential oil, fresh and smoldered volatiles of *O. kenyense*, *O. kilimandscharicum* and *O. lamiifolium* species, respectively. This study reports α-phellandrene chemotype of *O. lamiifolium* species' essential oil for the first time. In olfactometric tests, attraction of honey bees to smoldered, fresh and essential oil volatiles varied significantly ($p<0.05$) with mean values of attracted bees of 7.16 ± 0.22, 6.81 ± 0.28 and 4.56 ± 0.40, respectively. Smoldered volatiles of *O. kilimandscharicum*-Kirinyaga (OKI-KRN), *O. kenyense*-Nyeri (OKE-NYR) and *O. kilimandscharicum*-Nyeri (OKI-NYR) were more attractive as compared to other investigated volatiles, with respective mean values of attracted bees of 8.17 ± 0.17, 7.67 ± 0.21 and 7.17 ± 0.17. In field bioassay, smoldered volatiles of OKI-NYR and OKI-KRN were the most effective honey bee luring treatments with mean hive occupation times of 29 and 30 days, respectively. Synthetic blends of fresh and smoldered volatiles of OKI-NYR, OKE-NYR and OKI-KRN were prepared using major constituents identified in GC-MS analysis and. Subtraction bioassays of synthetic blends of smoldered volatiles of OKI-NYR, OKE-NYR and OKI-KRN were conducted to determine the specific chemical constituents that contributed to their respective bee attractant activity. A blend comprising of geraniol, nerol, neryl acetate, eucalyptol, estragole, (*E*)-β-ocimene, (*E*)-β-caryophyllene, (*E*)-β-farnesene and α-humulene (SBAC1) was identified as a promising lead towards formulation of a potent bee lure with MEC_{50} and MEC_{75} values of 6.9 and 15.4 µg/µl, respectively. Findings of this study have demonstrated that chemo-ecological interactions of honey bees and aromatic plants are very crucial in prompting bee hive colonization, which in turn has the potential to contribute towards food security, manufacturing and poverty reduction thus addressing some aspects of Kenya government's big four agenda.

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ABBREVIATIONS AND ACRONYMS

ASAL	Arid and Semi arid land
BWF	Fresh volatiles + bee wax
BWS	Smoldered volatiles + bee wax
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
FB	Full blend
GDP	Gross Domestic Product
JAICAF	Japan Association for International Collaboration of Agriculture and Forestry
KIK-NKF	*O. kilimandscharicum*-KRN + *O. kilimandscharicum*-NYR Fresh volatiles
KIK-NKS	*O. kilimandscharicum*-KRN + *O. kilimandscharicum*-NYR Smoldered volatiles
KEK-NKF	*O. kenyense*-NYR + *O. kilimandscharicum*-KRN Fresh volatiles
KEK-NKS	*O. kenyense*-NYR + *O. kilimandscharicum*-KRN Smoldered volatiles
KEK-NNF	*O. kenyense*-NYR + *O. kilimandscharicum*-NYR Fresh volatiles
KEK-NNS	*O. kenyense*-NYR + *O. kilimandscharicum*-NYR Smoldered volatiles
KNBS	Kenya National Bureau of Statistics
KRN	Kirinyaga County
KTBH	Kenya Top Bar Hive
LKP	Laikipia County
MEC	Minimum Efficative Concentration
NAFIS	National Farmers Information Service
NKU	Nakuru County
NYD	Nyandarua County
NYR	Nyeri County
OKE	*Ocimum kenyense*
OKI	*Ocimum kilimandscharicum*
OLA	*Ocimum lamiifolium*
SBAC	Synthetic Blend of Active Compounds
VCT	Volatile Collection Trap

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CHAPTER ONE

INTRODUCTION

1.1 Beekeeping in Kenya

Honey bees (*Apis mellifera*) are social insects which live in colonies and present a wide range of behaviours relying on olfaction (Seeley, 1999). For generations, they have been and still are the most important bees in man's honey hunting and beekeeping activities. They are widely reared due to their ability to nest in rock crevices and hollow trees (Crane, 1999). In Kenya, honey hunting and beekeeping has been practiced since time immemorial with some communities collecting honey from wild or traditionally managed colonies (USINKEN, 2013). In traditional beekeeping practice, bee colonies are kept in traditional bee hives, which included baskets, pots, gourds, hollow logs and rock crevices (Crane, 1999). Utilization of traditional bee hives allows large number of honey bees to establish their habitat in close proximity to human habitats thereby stabilizing honey production and crop pollination (JAICAF report, 2009).

Attempts to modernize Kenyan beekeeping sector were first made in 1950s by the colonial government where positions of a bee officer and a trainer were created to spearhead development in the sector (National beekeeping policy, 2009). From 1967 to 1971, government of Kenya through the support of Ox-Farm and Canadian International Development Agency (CIDA) conducted feasibility studies on viability of beekeeping in ASALs and established a national beekeeping project. In 1982, a national beekeeping station was established to provide training on beekeeping skills, new technologies, bee

equipment as well as dissemination of information to bee farmers (USINKEN 2013, National beekeeping policy, 2009).

Arid and semi-arid lands make 80% of Kenyan land and have a high potential of hive productivity due to abundance and diversity of bee flora (USINKEN, 2013). The beekeeping sector in Kenya is majorly a rural enterprise that improves livelihoods of local communities by ensuring food security, increasing household incomes, creating employment and conserving the environment as spelt out in Vision 2030 (Kiptarus and Asiko, 2012) and big four agenda. According to KNBS, 2009 census data, there are approximately 2 million bee hives in Kenya, majority of which are found in arid and semi-arid areas such as Eastern, Rift Valley, North Eastern and Coast regions (Table 1.1).

Table 1.1: Hive population in Kenya (KNBS, 2009)

Region	Number of hives
Nairobi	7,585
Central	95,972
Rift valley	706,765
Eastern	842,857
Western	36,765
Nyanza	48,124
North eastern	59,189
Coast	45,239
Total	**1,842,496**

Beekeeping enterprise requires minimal land, capital investment and labour while providing income from sale of a range of hive products such as honey, bee wax, propolis, pollen, bee venom, bee colonies, bee brood, queen bees and royal jelly (Hilmi *et al.*, 2011). Bee products are highly valued among beekeeping communities in Kenya due to their cultural importance in traditional ceremonies such as marriages where honey forms part of

dowry payment (FAO, 2009). Honey is also used traditionally as food, sweetener, medicine and preservative. It is also an important component of food that is rich in energy, vitamins, minerals, proteins and amino acids (National beekeeping policy, 2009).

1.2 Economic importance of beekeeping in Kenya

Increased demand for beehive products in food, cosmetic and pharmaceutical industries has made beekeeping to thrive as a commercial sector (Krell, 1996). Beekeeping is a valuable agricultural enterprise which produces approximately 25,000 metric tons of honey per annum and contributes about 4.3 billion Kenya shillings to GDP (The Hive Ltd, 2013). It is estimated that the Kenyan bee sector directly benefits approximately 547,440 people through sale of beehive products while indirectly supporting many others in beekeeping value addition chain, pharmaceutical, brewing, confectionery and cosmetics industries (Kiptarus and Asiko, 2012).

Introduction of modern beekeeping technologies has led to progressive growth in hive productivity although the current honey output estimated at 25,000 metric tons is far below Kenya's annual production potential of 100,000 metric tons (Kiptarus and Asiko, 2012). The country has been unable to meet its local market demand of honey and bee wax, hence the need for importation in order to bridge the deficit. Beekeeping industry has a great potential of earning foreign exchange, creating employment, conserving environment and increasing crop yield through pollination, which, is yet to be fully exploited (National beekeeping policy, 2009).

The immense ecological, agricultural and economic contribution made by honey bees through pollination is greatly underestimated (Muli *et al.*, 2014). Bees are particularly considered as the most important pollinators due to their high diversity of about 20, 000 species (Michener, 2007). They are fundamentally important agents of successful pollination which creates a significant economic and environmental impact (Kagio and Muriuki, 2018; Kelly, 2017). Globally, honey bees are the most predominant pollinators due to their ability to increase yield in a majority of crops with a contribution of approximately 153 billion Euros in annual economic value of crop pollination (Okinda, 2017; Gallai *et al.*, 2009; Klein *et al.*, 2007).

In Western Kenya, the economic worth of pollination of eight key crops currently stands at an approximate value of Ksh.320 million (Muli *et al.*, 2014). Beekeeping is possibly the only agricultural activity with an overwhelming positive impact on natural environment. Bees need plants to survive hence reduced tendency to cut trees in beekeeping areas encourages environmental conservation. Therefore, beekeeping is a valuable natural conservation tool which provides economic benefits from indigenous botanical resources in a non destructive manner (JAICAF, 2009). Beekeeping does not compete for space, light and resources with other farming activities but rather complements them through pollination services (The Hive Ltd, 2013).

King *et al*. (2009) invented a bee hive fence which has been shown to deter crop raiding elephants from accessing farms in human-elephant conflict hotspots in several studies. African elephants (*Loxodonta africana*) cause human deaths and injury; loss of crops (Parker *et al.*, 2007) and destruction of structures during crop raiding activities (Larmaque

et al., 2009). Beehive fence invention is based on a previous observation where elephants were found to avoid feeding on acacia trees hosting beehives in Zimbabwe as well as running away from the sound of agitated bees (King *et al.*, 2007; Karidozo and Osborn, 2005).

A bee hive fence is made by connecting a series of beehives with a wire allowing them to swing freely when disturbed by crop raiding elephants (King *et al.*, 2009). Elephants associate the sound of agitated bees to painful stings on sensitive body parts such as eyes, inner ears and trunk hence they run (King *et al.*, 2011). Alarm pheromone produced by agitated honey bees also serves as a deterrent to elephants. A study conducted in South African Greater Kruger National Park by Wright et al. (2018) showed that 25 out of 29 African bush elephants (*Loxodonta Africana*) responded to a synthetic blend of volatiles mimicking honey bee alarm pheromone by fleeing from a water hole on coming into contact with treated socks. Release of alarm pheromone by agitated bees serves as a deterrent to elephants when they come into contact with bee hive fence during crop raiding events.

Field trials conducted in Kenya have shown high success rate in elephant deterrence, improved crop yields and enhanced rural livelihoods through sale of honey and other hive products (King *et al.*, 2017). Use of beehive fences in areas that are prone to human elephant conflict has led to a reduction of human deaths, revenge killings on elephants, loss of crops and destruction of property (Okinda, 2016; Kairu, 2014; King *et al*., 2009).

Plate 1.1: A beehive fence used as a deterrent against crop raiding elephants (King et al., 2009).

1.3 Challenges facing beekeeping sector in Kenya

1.3.1 Low hive occupation

Climate change has impacted negatively on Kenyan bee sector leading to reduced water and bee forage sources associated with low hive occupation and high absconding rates. Decline in honey bee population associated with human instigated loss of natural flora (Kagio and Muriuki, 2018), excessive use of pesticides (Okeyo, 2017; Muli *et al*., 2014), and destruction of colonies during honey harvesting (Carroll, 2006) has been identified as a major cause of low hive occupation. Bee hive type has also been reported to influence occupation rates with Langstroth and Kenya Top Bar Hive (KTBH) (Figure 1.2), being

considered as the most and least attractive to bees, respectively (Kings *et al*., 2017; Carroll, 2006).

Plate 1.2: Kenya Top Bar Hives (KTBH) hanging on a tree

(Okeyo, 2017)

1.3.2 Lack of adequate research and hive management skills

Low hive occupation can be partly attributed to lack of knowledge on distribution of honey bee forage, swarming seasons in certain areas, pest management as well as sustainable harvesting (Carroll, 2006). Knowledge of floral calendar of their locality enables beekeepers to ensure higher occupation rates by conserving bee forage plants to ensure a constant nectar flow throughout the year (Namu *et al*., 2016). Traditional beekeepers in the local communities harbour a lot of information on bee flora calendar, bee luring techniques and suitable hive placement sites hence the need for an integrated approach towards high hive productivity. There is a high tendency of hives remaining unoccupied for a longer period of time if they are placed in unsuitable sites as well as during low forage season which coincides with low swarming season (National Beekeeping Report, 2009).

1.3.3 Diseases and pests

A recent survey on honey bee health status indicated presence of mites (*Varroa destructor)* Nosema causing fungi (*Nosema apis*) and three *Aflis* viruses that cause black queen disease in sampled hives in Kenya (Awino *et al.*, 2016). Varroa mites attack brood in combs as well as adults and is implicated for the spread of Nosema infection (Little *et al*., 2016). The infection is characterized by severe infection of intestines, bloody diarrhoea and deaths (Muli *et al*., 2014). The queen also loses ability to lay eggs and hence the colony disappears. Wax moths (*Galleria mellonella*) small hive beetles (*Aethina tumida*) and ants are common pests that infest bee hives in Kenya. The moths lay eggs in the hive where larvae feed on combs, wax and honey, eventually destroying an entire bee colony (The Organic Farmer, 2015).

1.3.4 Pesticides

There are reports of misuse of spray applications of pesticides in different countries resulting in contamination of nectar and pollen (Barnett *et al*., 2007; Kiljanek *et al*., 2016). Pesticides mainly neonicotinids are responsible for the global decline in honey bee population (Cresswell and Thompson, 2012). In addition to neonicotinids, other pesticides such as acaricides have also been found to have negative effects on health of honey bees (Hartz *et al*., 2010). Acaricides residues in bee hives are intentionally introduced by farmers into the hives to control Varroa mites (Chauzat *et al*., 2006). Increased probability of Nosema infection was reported in bees that consumed pollen with high fungicide contamination (Pettis *et al*., 2003).

1.4 Statement of the problem

A study by Namu *et al*. 2016 identified low hive occupation as one of the prominent challenge encountered by beekeepers in addition to pest attacks (Awino *et al.*, 2016, Muli *et al*., 2014 and The Organic Farmer, 2015), inadequate skills in hive management (Carroll, 2006), poor apiary conditions (National Beekeeping Report, 2009) and pesticides (Okeyo, 2017; Barnett *et al*., 2007). Low hive occupation poses a big threat to food security, livelihoods and economic developments of rural populations. A hive occupation status survey conducted in Kirinyaga and Kakamega Counties reported occupancy rates of between 30% and 70% whereas in Baringo County, occupation rates as low as 5% have been previously reported by Carroll (2006) and Gichora (2003).

Apart from decreased bee hive output, low hive occupation also contributes to decrease in crop yield associated with reduced pollination (Rhodes and Christopher, 2018) as well as rendering bee hive fence barriers ineffective against crop raiding elephants (King *et al*., 2017). The rising global demand for bee hive products, the need for enhanced crop pollination and reducing effectiveness of bee hive fences against crop raiding elephants necessitates initiation of prompt hive colonization. The success of sustainable beekeeping depends on possibility of establishment of a viable bee colony.

To initiate prompt hive colonization, some beekeepers in Mt. Kenya region traditionally use aromatic herbs and shrubs to smoke interior parts of a new beehive. Though the sweet scents emitted by selected *Ocimum* species is traditionally associated with their bee attractant activities, no chemo ecological studies on selected *Ocimum* species' volatiles have been reported in literature prior to this work.

1.5 Justification

Some plants contain highly specific semiochemicals that could attract honey bees to hives without encouraging pest infestation (Ande *et al.,* 2008). Although the role of herbs and shrubs in bee keeping remains poorly understood and appreciated by local beekeepers, the potential held by indigenous knowledge in the future of beekeeping sector in Kenya cannot be underestimated (African Union Inter-African Bureau for Animal Resources, 2016; JAICAF beekeeping report, 2009).

Based on indigenous knowledge from Mt. Kenya region, essential oils, fresh and smoldered volatiles and synthetic blends of chemical constituents of *Ocimum kenyense, O. kilimandscharicum* and *O. lamiifolium* species were investigated for their chemical composition and their potential to lure honey bees into hives. Findings of this study are expected to provide a scientific basis of traditional use of *Ocimum* species as honey bee swarm lures.

Possibility of integration of indigenous bee luring knowledge into modern beekeeping practices should be considered as a way of boosting hive colonization rates. Prompt hive colonization will improve hive output, increase crop pollination and enhance efficiency of bee hive fences among other benefits. In addition to direct application of *Ocimum* species as bee attractants, possibility of cultivation and development of commercial bee attractants based on indigenous knowledge could be explored.

1.6 Objectives

1.6.1 General objective

To establish the potential of essentail oil, fresh and smoldered volatiles from selected *Ocimum kenyense, O. kilimandscharicum* and *O. lamiifolium* as honey bees (*Apis mellifera scutellata*)

1.6.2 Specific objectives

i. To identify and compare the chemical constituents of essential oil, fresh and smoldered volatiles of *Ocimum kenyense, O. kilimandscharicum* and *O. lamiifolium* species.

ii. To determine the differences in attraction of honey bees (*Apis mellifera scutellata*) to olfactory cues of selected *Ocimum* species' volatiles.

iii. To undertake subtraction bioassays and formulate synthetic blend(s) of identified compound(s) that contribute(s) to honey bees' attraction to selected *Ocimum* species.

1.7 Hypotheses

i. Chemical compositions of selected *Ocimum* species' essential oil, fresh and smoldered volatile blends do not vary with species and agro ecological zone of origin

ii. Honey bee attraction to selected *Ocimum* species essential oils, fresh and smoldered volatile blends do not vary with species and agro ecological zone of origin

iii. Synthetic blends of certain specific constituents can be formulated using commercial standards for possible upscale as bee lure

1.8 Limitations and scope of the study

This research work was limited to only a few selected *Ocimum* species namely *Ocimum kenyense, Ocimum kilimandscharicum* and *Ocimum lamiifolium*, each of which were sampled from different agro-ecological zones: Laikipia-Nanyuki, Nyeri-Kiganjo, Kirinyaga-Sagana, Nyandarua-Ol jororok and Nakuru-Bahati (Baker, 1967; Jaetztold *et al*., 2006; Republic of Kenya: Nakuru County, 2013) (Appendix 1). Essential oil, fresh and smoldered volatiles of aerial parts of *O. kenyense, O. kilimandscharicum* and *O. lamiifolium* species were studied for their potential to attract honey bees (*Apis mellifera scutellata*) foragers. Laboratory and field experiments were conducted at the Center for African Medicinal and Nutritional Flora and Fauna (CAMNFF) in Masinde Muliro University of Science and Technology (MMUST), Kakamega County.

CHAPTER TWO

LITERATURE REVIEW

2.1 Honey bees

Approximately 20,000 species of bees have been identified in the world (Hammond and Blankenship, 2009). All honey bees belong to *Hymenoptera* order, *Apoidae* family and *Apis* genus and are characterized by their abilities to build combs from wax, produce and store honey (Gupta *et al*., 2014). There are forty-four sub-species of honey bees in genus *Apis*, twenty-six of which are distributed in different parts of Africa (Gupta *et al.*, 2014). There are three distinct groups of bees in genus *Apis* namely cavity nesting bees (*A. mellifera, A. cerana* and *A. keschevnikov*), giant bees (*A. dorsata, A. nigrocinta* and *A. laboriosa*) and dwarf bees (*A. florea* and *A. andreniforms*) (Han *et al*., 2012; Raffudine and Cruzier, 2007; Arias and Sheppard, 2005).

According to Hammond and Blankenship (2009), honey bee species (*A. mellifera*) is native to Africa, Western Asia and Europe, though human introduction of the species to Eastern Asia, America and Australia occurred in 17th century. Apart from *A. mellifera* all the other honey bee species are confined to Asia. In Kenya, *Apis mellifera* has been identified as the most important honey bee species with exist four races namely, *Apis mellifera scutellata, Apis mellifera yemenitica, Apis mellifera littorea* and *Apis mellifera monticola* whose distribution is shown in Figure 2.1 (Carroll, 2006).

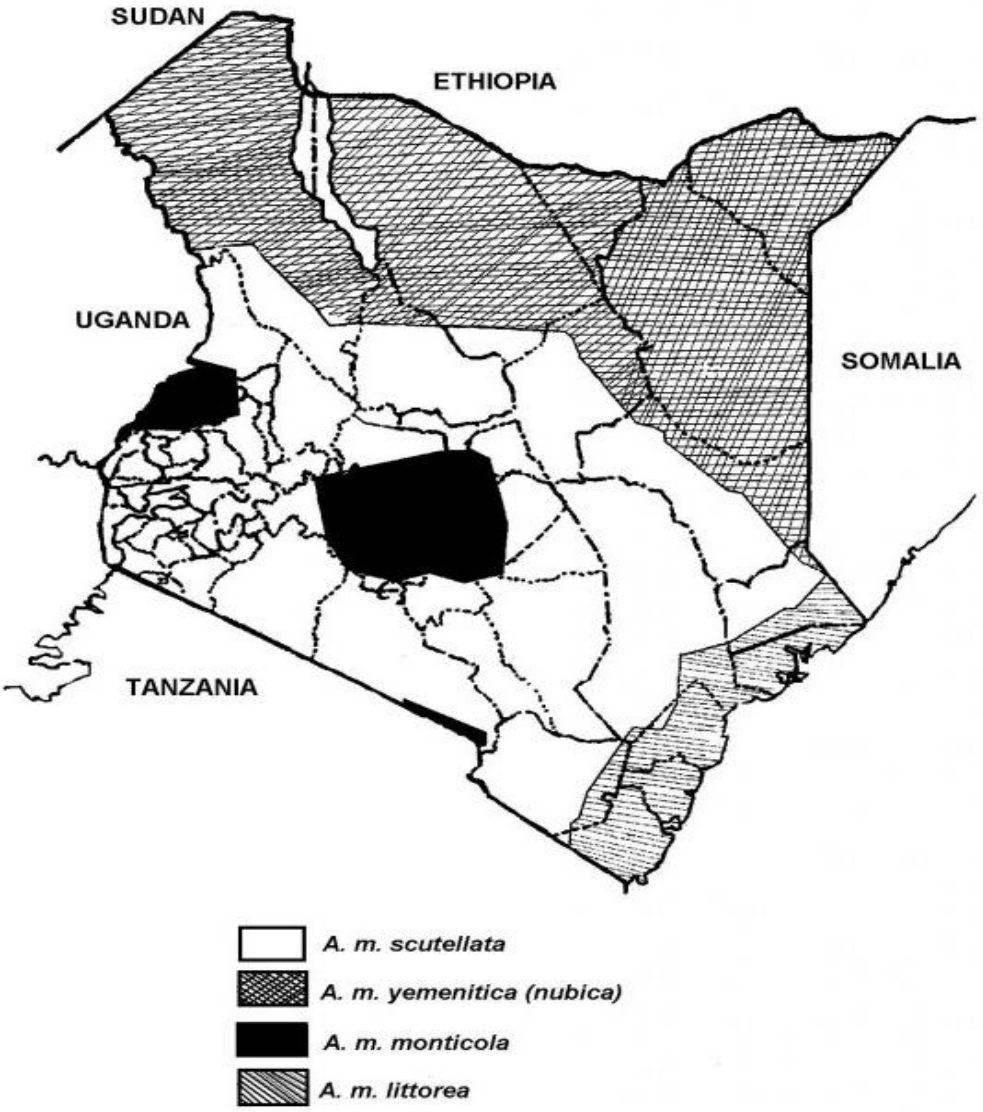


Figure 2.1: Map of distribution of *A. mellifera* species in Kenya (Carroll, 2006)

2.2 Morphology of honey bee species in Kenya

Carroll (2006) gives a morphological and behavioral description of honey bees (*A. mellifera*) species in Kenya (Figure 2.2 A-D). The small bee with a brown body and black stripes which has high reproductive and migratory rates is identified as *A. mellifera scutellata.* It is a highly aggressive bee whose swarm can nest in a broad range of sites

from open nests to cavities. The bee is commonly found in Savannah grasslands. A large, dark and gentle with longer hairs than other races are mountain bees found in Meru and Mount Elgon is identified as *A. mellifera monticola*. It is less productive and non-migratory. It tends to reduce brood rearing upon sensing forage decline.

A coastal lowland bee with no migratory tendencies which rears brood throughout the year due to availability of forage is identified as *A. mellifera littorea.* The mall bee with a slender stomach with large yellow colour band is found in arid areas of Northern Kenya are identified as *A. mellifera yemenitica*. It adapts to drought by excessive migration in order to survive. Morphological differences of *A. mellifera* sub-species are shown in Plate 2.1: A, B, C and D (Carroll, 2006).

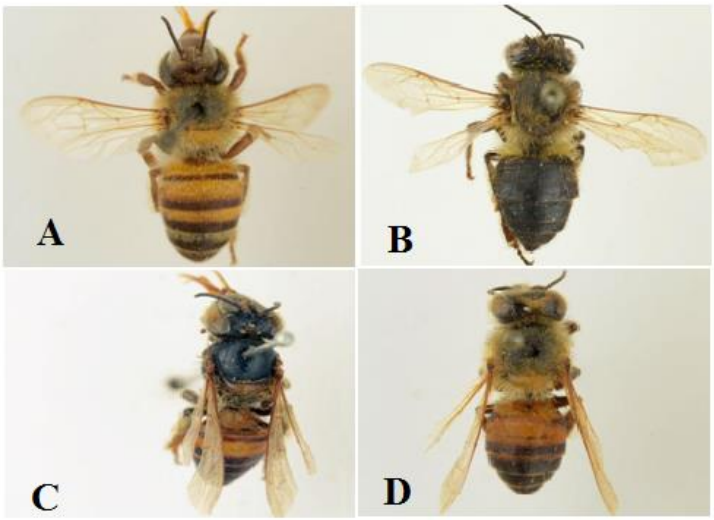


Plate 2.1: Morphology of *A. mellifera scutellata* (A), *A. mellifera monticola* (B), *A. mellifera littorea* (C) and *A. mellifera yemenitica* (D)
(Carroll, 2006)

2.3 Chemical communication in honey bees (*A. mellifera*)

Chemical communication between individuals and castes in a colony is an important area of physiology of honey bees. The highly complex social organization which maintains integrity and function of honey bee colony is mediated through chemical communication involving pheromones (Trhlin and Rajchard, 2011). Pheromones are chemical substances secreted by animals' exocrine glands and elicit behavioural or physiological response from members of the same species (Bortoletti and Costa, 2014).

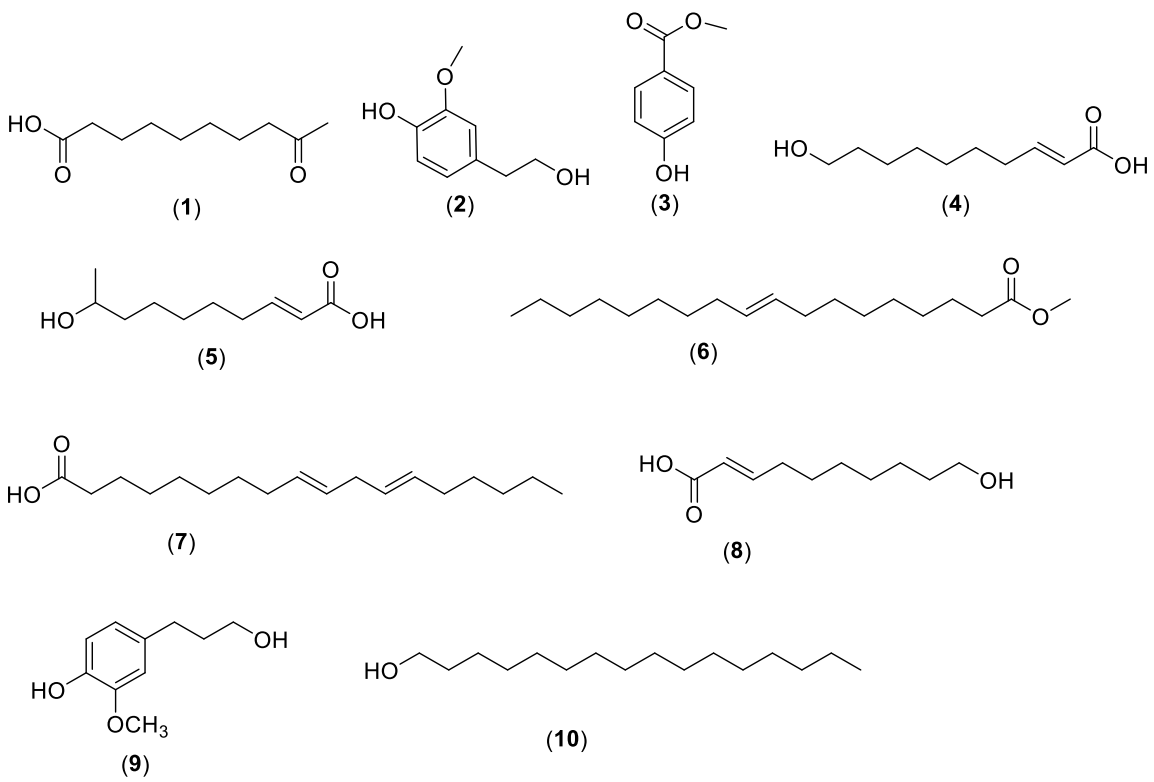
The chemicals are transmitted by direct contact as a liquid or as a vapour and elicit defensive, flight, mating, feeding and aggregation responses from the recipient (Slessor *et al.*, 2005). The effect of a pheromone on a recipient can be classified as releaser, primer or both. Releaser pheromones cause a rapid change in the recipient's behaviour while primer pheromones act slowly and have long term effects on physiology and behaviour of the recipient (Trhlin and Rajchard, 2011). Pheromones are involved in almost every aspect of honey bees' colony life such as development and reproduction, foraging, defense, orientation and integration of colony activities (Bortoletti and Costa, 2014).

Honey bee pheromones allow communication among all bee castes such as queen-worker, worker-worker, queen-drones and brood-worker. The chemical signals released by a queen, worker and brood elicit various behavioural and physiological responses in other bees of the same colony. Queen pheromones exhibit both releaser and primer effects whereas worker and brood pheromones exhibit releaser and primer effects, respectively (Trhlin and Rajchard, 2011).

2.3.1 Honey bee (*A. mellifera*) pheromones

Queen mandibular pheromone (QMP) has primer effects by enhancing brood care by slowing workers' transition from nursing to foraging as well as regulating colony swarming and queen rearing behaviour (Wanner *et al.*, 2007). When the colony size increases, the amount of QMP getting into contact with individual bees decreases hence queen rearing begins in preparation for swarming (Trhlin and Rajchard, 2011). QMP comprises of five major compounds namely; 9-oxo-2-decenoic acid (9-ODA) [**1**], 4-hydroxy-3-methoxyphenyl ethanol (homovanillyl alcohol) [**2**], methyl-*p*-hydroxybenzoate (HOB) [**3**], *cis*-9-hydroxydec-1-enoic acid (9-HDA) [**4**] and *trans*-9-hydroxydec-1-enoic acid (9-HDA) [**5**] (Hoover *et al.*, 2003).

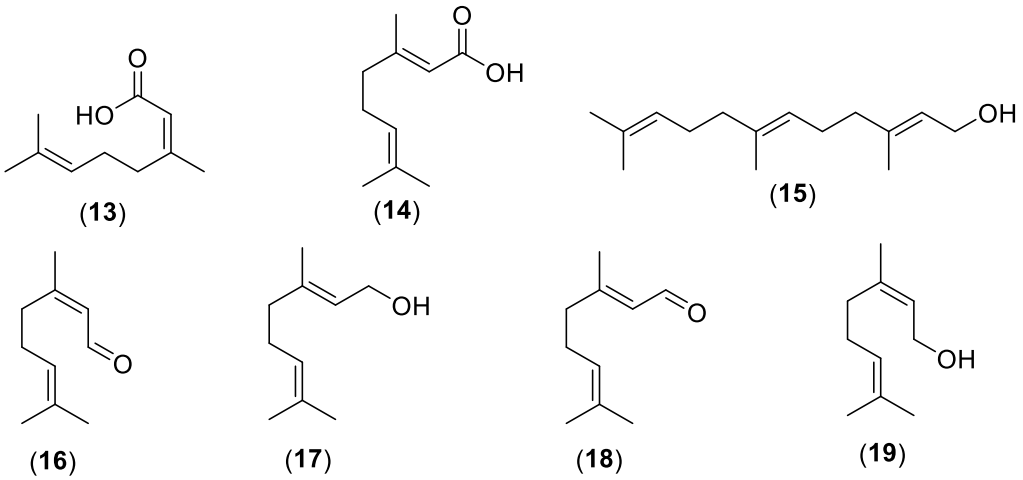
Queen retinue pheromone (QRP) acts a releaser and primer pheromone by attracting workers to attend the queen and by inhibiting development of worker ovaries rendering them infertile, respectively (Wanner *et al*., 2007). During mating, a virgin queen releases sex pheromones to attract drones into a mating flight dominated by 9-ODA (**1**) (Brockmann *et al*., 2006). The full queen retinue pheromone response is achieved when all the five major components of QMP are present in addition to synergistic compounds namely; methyl oleate [**6**], linoleic acid [**7**], (2*E*)-10-hydroxydecenoic acid (10-HDA) [**8**], coniferyl alcohol [**9**] and hexadecane-1-ol [**10**], (Hoover *et al.*, 2003).



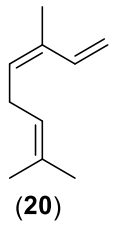
The role of workers in a bee colony is to provide defense of the nest, brood and stored food. Defensive behaviour of worker bees is controlled by alarm pheromone. The alarm pheromone whose main component is isopentyl acetate (IPA) [**11**] is produced by sting apparatus of a worker (Breed *et al.*, 2004) while its mandibular glands produce 2-heptanone (2-HPT) [**12**], which has a lower ability in recruiting workers to sting as compared to IPA. Due to its repellent properties, 2-HPT is used to mark flowers depleted of nectar and pollen causing foragers to avoid flowers recently visited or rejected by other bees (Goulson and Stout, 2001; Giurfa, 1993).

Honey bee workers also produce Nasonov pheromone which is also used to mark nest entrance, orient lost bees and mark rewarding food and water sources (Wells *et al.*, 1993).

Chemical constituents of Nasonov pheromone include nerolic acid [**13**], geranic acid [**14**], (*E, E*)-β-farnesol [**15**], geranial [**16**], geraniol [**17**], neral [**18**] and nerol [**19**] (Free and Pickett, 1981). Apart from eliciting clustering activity in a colony during swarming, Nasonov pheromone orients bees to a new nesting site (Abdullah *et al*., 1991). During swarming, workers crowd around the queen and release nasonov pheromone to attract other bees to that cluster (Janson *et al*., 2005).



(*E*)-β-Ocimene [**20**] is a volatile brood pheromone emitted by honey bee brood to control foraging activity in workers. Young larvae (brood) produce more (*E*)-β-ocimene [**20**] to promote foraging activity since their need to be nursed is low (Maisonnasse *et al.*, 2010). Emission of high quantities of (*E*)-β-ocimene [**20**] increases foraging activity of workers in an attempt to accumulate food reserves (Le Conte *et al.*, 2001).



2.3.2 Honey bee (*A. mellifera*) pheromone mimicry in plants

Floral scents serve as attractants to species specific pollinators whereas foliar scents tend to protect plants by deterring herbivores and attracting natural enemies of those herbivores (Pichersky and Gershenzon, 2002). Several studies have indicated that some plant species also mimic honey bee pheromones not only to attract pollinators but also as a defense against impending attacks by phytophagous insects and herbivores (Armengol *et al.*, 2017; Navia-Gine *et al.*, 2009). Several studies have indicated existence of phenomenon of honey bee pheromone mimicry in some plant species.

Lemon grass (*Cymbopogon citratus*) which is used traditionally to lure honey bees into hives probably communicates with honey bees by mimicking Nasonov pheromone emitted by workers to orient a colony to a nesting site or a rewarding source of food (Trhlin and Rajchard, 2011). Occurrence of geraniol [**17**], neral [**18**] and nerol [**19**] in *Cymbopogon citratus* essential oil indicates existence of possible Nasonov pheromone mimicry by lemon grass (Gbenou *et al.*, 2013; Tajidin *et al.*, 2012; Matasyoh *et al.*, 2011). Japanese orchid (*Cymbidium floribundum*) is attractive to honey bee (*Apis cerana japonica*) and hence it is traditionally used to attract bee swarms. Before pollination, *C. floribundum* flowers emit a volatile, (2*E*)-10-hydroxy-2-decenoic acid (10-HDA) [**8**] that mimics Asian honey bee (*Apis cerana*) queen mandibular gland secretion (Johnson and Schiestl, 2016; Sugahara *et al.*, 2013).

A rare plant species, *Euphorbia flavicoma* in Mediterranean shrub lands emit high quantities of (*E*)-β-ocimene [**20**] so as to prompt foraging activity in bees. This adaptation enables the rare species to compete for pollinators with dominant Rosemary plant species

(*Rosmarinus officinalis)* (Fillela *et al.*, 2013). Although honey bees and bumble bees are known to be attracted by (*E*)-β-ocimene [**20**], no behavioural studies have been done to demonstrate its role in pollinator attraction (Armengol *et al.*, 2017).

2.4 Bee attractants

Bee attractants are defined as chemical signals that influence the selection of potential nest cavities by honey bee swarms or products that are designed to increase bee visitation to treated crops with an aim of increasing pollination (Schmidt, 2001). Recent advances in understanding of pheromones, synthesis and manipulation of these chemicals have given beekeepers and farmers new tools to boost hive occupation and crop pollination. In general, use of bee attractants is required when conditions are not optimal for pollination; the crop is less attractive to bees; as well as to orient bee swarms to specific nesting sites during swarming season (Vita bee health, 2013; ICB Pharma-Crop Solution, 2018).

The idea of application of attractants is to focus bees away from competing blooms and other nesting sites while improving their foraging efficiency and tendency to settle in a certain site. As much as bee attractants encourage hive scouting and floral visitation, they do not necessarily lead to nesting and pollination if conditions are unsuitable. For instance, if there are no bees in the area, no chemical attractant will draw them from great distances. Similarly, if the flowers are unattractive, no attractant will make pollination to occur (Delaplane *et al*., 2000).

2.4.1 Conventional bee attractants

Conventional bee attractants are made up of blends of chemical constituents just like pheromones, plant volatiles, flower oils, sugar and proteins that mimic insects' attraction systems found in nature. Apart from being used to trap harmful insects, attractants can also be used to attract beneficial insects such as honey bees for enhanced honey production and crop pollination (ISCA, 2019).

2.4.2 Bee pheromone based attractants

A number of bee attractants available in the market have been formulated using synthetic chemical components that mimic honey bee pheromones. Synthetic bee attractants improve pollination of sprayed crops by reducing competition from attractive flowers in the neighbourhood. They can also be used to manipulate bee behaviour by encouraging foraging and settlement in new nesting sites (Elis and Delaplane, 2009). Some of the commercially available bee lures are formulated on basis of Nasonov, queen mandibular and brood pheromones and are marketed under trademarks such as SuperBoost™, FruitBoost™, BeeScent™, Beeline™, Bee Here™ and SwarmLure™ (Lait *et al.,* 2012; Sivaram and Jayaramappa, 2012; Elis and Delaplane, 2009).

Fruit Boost™ is formulated on the basis of major components of queen mandibular pheromone (QMP). When sprayed on crops in blooming stage, Fruit Boost™ increases foraging activity of honey bees, resulting in more floral visits and elevated rates of pollination. The attractant can also be used to lure bee swarms into a new nesting site since QMP elicits clustering activity during swarming (Elis and Delaplane, 2009).

BeeScent™, Beeline™, BeeHere™ and SwarmLure™ attractants mimic Nasonov pheromone. The attractants can be used to attract and capture swarms by beekeepers (Delaplane *et al.,* 2000). A study on influence of Bee Scent™ on pollination and yield parameters in guava (*Psidium guajava* L), revealed enhanced length; diameter and weight of fruit in guava (Sivaram and Jayaramappa, 2012).

SuperBoost™ encourages foragers to visit more flowers and collect more pollen and nectar by mimicking brood pheromone. The attractant benefits a colony through production of more brood and honey hence increasing the potential of swarming. Increase in flower visitation, pollen collection and adult bee population benefits crops in close proximity to hives through pollination (Lait *et al.,* 2012).

2.4.3 Essential oil based attractants

Vita™ and Biopolin™ swarm attractants are based on several natural compounds such as constituents of essential oils. The constituents of essential oils are carefully selected in proportions which enable maximum possible synergy in form of attracting swarms to hives and crop fields. Swarm attractant swipes impregnated with natural oils of Vita™ has been shown to attract 60-90% of swarms since its introduction in 2011 (Vita bee health, 2013). The chemical constituents of the natural essential oils are protected by a patent. The product helps beekeepers to manage their swarms by orienting them to occupy convenient locations thus increasing prospects of bigger honey harvests.

Plate 2.2 A and C show Vita™ wipes on hive and a twig while Plate 2.2 B and D show populations of bees colonizing the hive and the twig after baiting. Biopolin™ attractant is

a slow release technology product which applied once at the beginning of crop flowering season. The product prevents bees from visiting neighboring wild plants which may be more attractive than the crops in need of pollination. Crop yields have been found to increase by 10-25% up on application of Biopolin™ (ICB Pharma-Crop Solution, 2018).

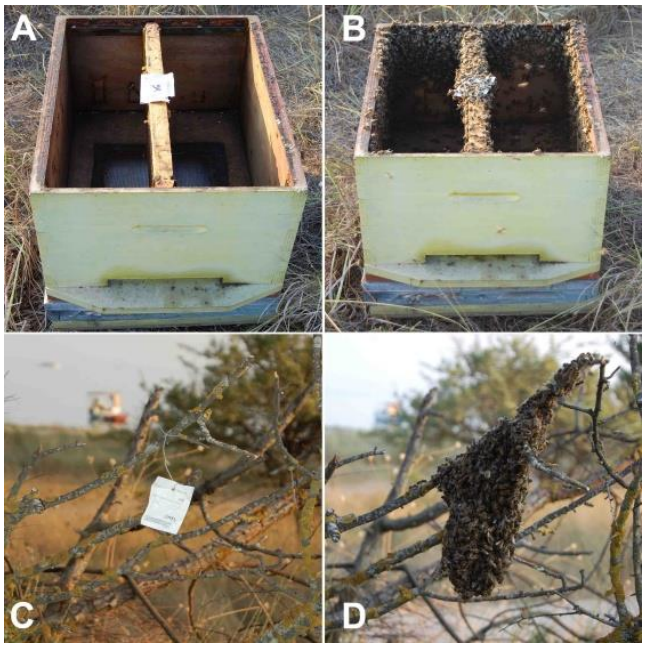


Plate 2.2: Use of Vita™ wipes to attract honey bees to a hive (A and B) and a twig (C and D) (Vita bee health, 2013)

2.4.4 Indigenous bee attractants

Since hive colonization is not guaranteed, traditionally, bee keepers use baits such as wax, slum gum, honey, propolis and a concoction of herbs to attract bees to occupy new hives (Babarinde *et al.*, 2015; Ande *et al.*, 2008). However, studies have shown that pre-colonization pest infestation by sugar ants (*Camponotus consobrinus*) and waiver ants (*Oecophylla langinoda*) tends to occur in slum gum, bee wax, honey and propolis baited

hives (Babarinde *et al.*, 2015). Use of aromatic plant species as honey bee lures is part of traditional beekeeping practices that is widespread in different parts of the world (JAICAF, 2009).

In Uganda, bees are baited by smoking or rubbing hives with lemon grass (*Cymbopogon citratus*) (Muli and Fraizer, 2011). Ethiopian beekeepers traditionally fumigate new hives using smoke of two or more bee baiting plants and bee wax. Some of the commonly used fumigant plant species are *Ekebergia capensis, Orostegia intergrifolia, Syzygium guineense, Juniperus procera* and *Boswelia papifera* (Abebe, 2011; Bogale, 2009).

Several plants of Lamiaceae family such as *O. kilimandscharicum* and *Plectranthus* species are traditionally used to scent new hives and attract bee colonies according to JAICAF Report (2009). In Eastern Kenya, the Kamba community bait new hives before placement using leaves of *O. kilimandscharicum* and *O. basilicum* (Wambua, 2015). Use of *O. gratissimum*, *O. sanctum* and *O. suave* as traditional bee lures have been described in Nigeria (Ande *et al.*, 2008), Ethiopia (Abebe, 2011) and Tanzania (Ntenga and Mugongo, 1991), respectively.

2.5 The genus *Ocimum*

Plants of genus *Ocimum* are aromatic annual and perennial herbs and shrubs of Lamiaceae family native to tropical America, Asia and Africa (Chowdhury *et al.,* 2017). General characteristics of *Ocimum* plants include a square stem, opposite and decussate leaves with many gland dots. The plants' flowers are small, anterior corolla lips are flat with stamens and style extending upward towards posterior lip of corolla (Hiltunen and Holm, 2003).

There are more than 150 *Ocimum* species in the world with over 50 and 14 species being identified in Africa and Kenya, respectively (Paton *et al.*, 1999).

2.5.1 Occurrence of selected three *Ocimum* species used in this study

The selected *Ocimum* species in this study are distributed in different habitats within the country. For instance, *O. lamiifolium* is found at an altitude of 1000-2100 metres above the sea level in forest clearings, wetlands, secondary bush and forest edges (Bentjee, 1994). *Ocimum kilimandscharicum* occurs in grasslands and disturbed grounds at an altitude of 1000-2000 metres while *O. kenyense* occurs in wet places or seasonally water logged grass lands at an altitude of 1200-2000 metres (Paton *et al*., 1999).

2.5.2 Botanical features of the three selected *Ocimum* species

Botanical features of selected *Ocimum* species have been described by Bentjee (1994) and Paton *et al.*, (1999). *Ocimum kenyense* species is an herb growing to 0.3 metres high, leaves erect and petioles up to 3 mm long is identified as in Plate 2.3 while *Ocimum kilimandscharicum* species is a shrub up to 2 metres high, leaves spreading and petioles more than 3 mm long (Plate 2.4). *Ocimum lamiifolium* is a shrub up to 3 metres high, flowering calyces 4-5 mm long, petioles of lower leaves shorter than blades and blades ovate is identified as species (Plate 2.5).

Plate 2.3: A habil of *Ocimum kenyense* Ayobangira plant species specimen

(Paton *et al*., 1999)

Plate 2.4: A habil of *Ocimum kilimandscharicum* Guerke plant species specimen

(Paton *et al*., 1999)

Plate 2.5: A habil of *Ocimum lamiifolium* Damakese plant species specimen

(Paton *et al*., 1999)

2.6 Essential oil volatiles of the genus *Ocimum*

Essential oils are natural products generally present in varying concentrations in specialized structures such as glandular trichomes and oil cells of foliar and floral plant parts (Bakkali *et al*, 2008; Simon *et al*., 1999). Chemical compositions of Basil essential oils have been studied since 1930s. The term Basil is used to collectively refer to plants of *Ocimum* genus. Basil oil consists of monoterpenes, sesquiterpenes, oxygenated terpene derivatives, esters and aldehydes (Hiltunen and Holm, 2005).

These oils have attracted a lot of attention from researchers worldwide due to their great potential as commercial sources of natural flavours, perfumes, medicine and repellents. However, very few studies have focused on chemistry of fresh and smoldered volatiles of *Ocimum* species. Chemical profiles of head space volatiles give a realistic picture of plants' aroma when freshly trapped, smoldered or thermally expelled from aerial parts such as flowers, leaves and fruits (Tholl *et al.*, 2006).

2.6.1 Chemo types of *Ocimum* species' essential oils

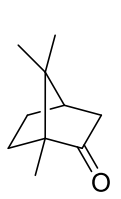
Chemo types of essential oils are derived from plants that are phenotypically similar (same species) but are chemically different in terms of chemical constituents present. Wild cross pollination, altitude, climate and other environmental factors lead to existence of essential oil chemo types in *Ocimum* species. Chemo typing of *Ocimum* species has been defined in terms of all major chemical components constituting more than 20% of the essential oil of the plant (Grayer *et al.,* 1996). Variations in chemo types of some *Ocimum* species are shown in table 2.1.

Table 2.1: Variations in chemo types of some *Ocimum* species

Plant species	Location	Major compounds	Chemo type
O. kilimandscharicum	Kenya	Camphor [**21**] (70.43%), Eucalyptol [**22**] (7.2%), limonene [**23**] (6.23%), camphene [**24**] (5.07%) (Bekele and Hassanali, 2001).	Camphor
	Nigeria	Methyl eugenol [**25**] (40.4%), borneol [**26**] (11.9%), linalool [**27**] (10.6%) (Oladipupo *et al.*, 2014).	Methyl eugenol
	USA	Linalool [**27**] (41.94-58.85%), camphor [**21**] (17.02-15.82%), eucalyptol [**22**] (10.18-6.38%) (Charles and Simon, 1992).	Linalool
O. lamiifolium	Tanzania	Bornyl acetate [**28**] (30.3%), *o*-cymene [**29**] (11.4%), camphene [**24**] (5.9%) (Runyoro *et al.*, 2010).	Bornyl acetate
	Ethiopia	Sabinene [**30**] (31.28%), α-phellandrene [**31**] (13.34%), 3-octen-1-ol [**32**] (13.42%) (Kifle *et al.*, 2007).	Sabinene
	Cameroon	Sabinene [**30**] (33.8%), (*Z*)-β-ocimene [**33**] (8.4%), terpinen-4-ol [**34**] (8.4%), (*E*)-β-caryophyllene [**35**] (5.1%) (Tchoumbougnang *et al.*, 2006).	Sabinene
O. kenyense	Kenya (Nairobi)	Eucalyptol [**22**] (36.93%), β-selinene [**36**] (23.07%), estragole [**37**] (12.86%), iso eugenol [**38**] (8.23%) (Bekele and Hassanali, 2001).	Eucalyptol-selinene
	Kenya (Ngong)	Eucalyptol [**22**] (38%), estragole [**37**] (24%) (Mwangi *et al.*, 1994)	Eucalyptol-estragole
O. gratissimum	Kenya	Eugenol [**42**] (68.8%), methyl eugenol [**25**] (13.2%) (Matasyoh *et al.*, 2008).	Eugenol
	Cameroon	Terpinene [**39**] (21.90%), α-phellandrene [**31**] (21.10%), limonene [**23**] (11.40%), thymol [**40**] (11.20%) (Tchoumbougnang *et al.*, 2005).	Terpinene-phellandrene
	Rwanda	Thymol [**40**] (35.40%), *p*-cymene [**41**] (18.30%), eugenol [**42**] (10.70%) (Ntezurubanza *et al.*, 1987).	Thymol

Table 2.1 Continued.

Plant species	Location	Major compounds	Chemo type
O. basilicum	Kenya	Camphor [**21**] (31.0-32.6%), linalool **[21]** (28.2-29.3%) (Dambolena *et al.*, 2010).	Camphor-linalool
	Nigeria	Estragole [**37**] (60.30%), linalool [**27**] (10.00%) (Kasali *et al.*, 2005).	Methyl chavicol
	Egypt	Linalool [**27**] (44.18%), eucalyptol [**22**] (13.65%), eugenol [**42**] (8.59%) (Ismail, 2006).	Linalool



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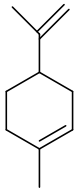
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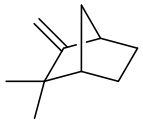
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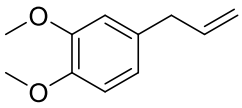

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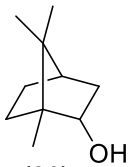

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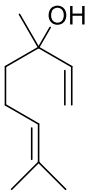

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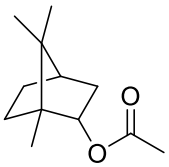

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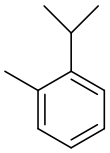
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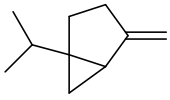
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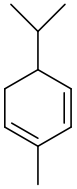
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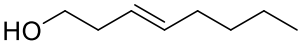

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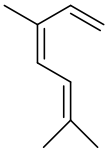
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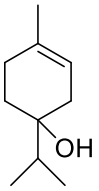

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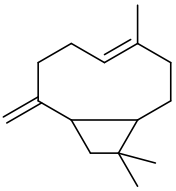
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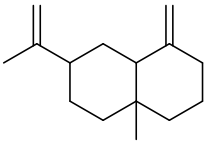
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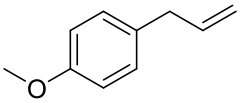

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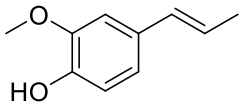

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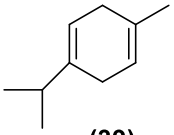
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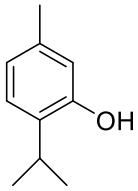

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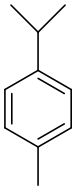
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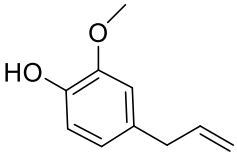
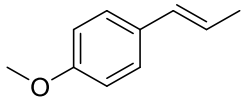


(42)

2.6.2 Factors that influence chemical profiles of *Ocimum* essential oils

Extracted essential oils differ in quality and quantity depending on extraction technique (Benitez *et al*, 2009), genetic makeup, plant part used (Narendra *et al.*, 2009) and agro-climatic conditions (Dey and Chodhuri, 1983). A comparative analysis of steam distilled and hydro distilled essential oils revealed that steam distillation extraction method yields more chemical constituents (Vani *et al*., 2009).

Genetic variations are known to contribute to differences in chemical profiles observed in essential oils of different species of the same genus (Graven *et al.*, 1990). Interspecific chemical variations among *Ocimum* species have been observed in several studies. For instance, analysis of steam distilled essential oils of four Brazilian *Ocimum* species led to identification of anethole [**43**] (52.2%), (*Z*)-methyl cinnamate [**44**] (29.4%), linalool [**27**] (44%) and eugenol [**42**] (64.11%) as the major compounds in *O. selloi, O. americanum, O. basilicum var. minimum* and *O. micranthum*, respectively (Vieira *et al.*, 2014).



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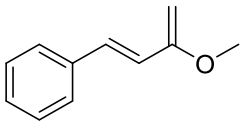
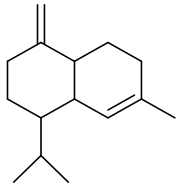


(44)

The *Ocimum* essential oils' chemical profiles have also been found to vary with plant part used. GC-MS analysis of *O. kilimandscharicum* species' floral and foliar essential oils from Nigeria led to identification of methyl eugenol (40.4%), borneol (11.9%) and linalool (10.6%) as major components of floral oil; while foliar oil was rich in methyl eugenol [**25**] (53.9%) and γ-cadinene [**45**] (16.2%) (Oladipupo *et al.*, 2014). Chemical variations in *O.*

basilicum stem, foliar and floral essential oils' estragole [**37**], limonene [**23**] and *p*-cymene [**41**] content were also reported by Chalchat and Ozcan (2008).



(45)

Variations in yield and composition of essential oil of the same plant species grown under different agro-climatic conditions have also been reported. Several factors such as solar irradiation, temperature (Chang, 2005); rainfall (Pushpangadan and George, 2012) and soil type (Burdina and Priss, 2016) among others contribute significantly to variations in essential oil quality and quantity. High rainfall, humidity, long days and high temperature conditions also promote *Ocimum* growth and oil production (Pushpangadan and George, 2012). Chang (2005), observed a threefold essential oil yield of *O. basilicum* at warm temperature of 25 ˚C.

Plants of genus *Ocimum* flourish under a variety of soil conditions ranging from rich loam, poor lateritic, saline, alkaline to moderately acidic. Increase in soil salinity not only increased the essential oil yield but also influenced the quantities of individual chemical components. Tarchoune *et al.* (2013) reported a decrease in *O. basilicum*'s eugenol [**42**] content under high salinity conditions and an increase in its methyl eugenol [**25**] content under low salinity conditions possibly due to methyl transference.

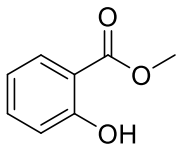
2.7 Fresh and smoldered volatiles of the genus *Ocimum*

Fresh plant volatile emissions comprise of highly volatile constituents which are released into the surrounding air under natural environmental conditions while essential oils comprise of chemical constituents that are released on exposure to steam. Head space sampling gives a more realistic picture of volatile profile emissions detected by insects hence its most preferred for chemo ecological studies (Tholl, 2006). Steam distillation enhances release of less volatile constituents that appear in chemical profiles of essential oils though they may be absent in corresponding fresh volatiles' profiles (Prabhu *et al*., 2009).

Currently, there is limited documented literature on chemical compositions of fresh and smoldered volatiles of *O. kenyense*, *O. kilimandscharicum* and *O. lamiifolium* species. However, in related studies, chemistry of fresh and smoldered volatiles of other species of genus *Ocimum* has been studied and documented. Tarchoune *et al*., (2013) and Yousif *et al.,* (1999) reported chemical compositions of fresh and dried volatiles of *O. basilicum,* respectively; while Fatope *et al.,* (2008) reported its essential oil chemical composition. Remarkable differences in chemical compositions of fresh volatiles and essential oils of *O. basilicum* were observed with linalool [**27**] and estragole [**37**] being identified as major constituents, respectively (Fatope *et al.,* 2008; Tarchoune *et al*., 2013).

Dekker *et al.,* (2011), reported chemical composition of Eritrean *O. forskolei* species fresh volatiles; while Ali *et al.* (2017) and Fatope *et al.* (2008) reported chemical composition of essential oils of Oman and Yemen *O. forskolei* species. (*E*)-β-ocimene [**20**] and methyl

salicylate [**46**] were identified as major constituents of fresh and essential oil volatiles of *O. forskolei* respectively. Dube *et al.* (2011) collected and analyzed smoke volatile constituents of *O. lamiifolium*. However, they observed that "the smoke leaf extracts resulted in highly complex chromatograms that were difficult to interpret" hence they could not describe the chemical profile of highly mosquito repellent smoke.



(**46**)

2.8 Biological activities of selected *Ocimum* species

2.8.1 Medicinal activity

Traditionally, *O. kilimandscharicum* and *O. kenyense* species are used as pain relievers. Mwangi *et al.*, (2012) reported significant antinoceptive activity in animal models hence validating the species' traditional use. Essential oil of *O. kilimandscharicum* species has been used to formulate Naturub^{TM} balm is registered by the Pharmacy and Poisons Board of Kenya (Ligare, 2010). The major chemical constituent of Naturub^{TM} balm is camphor [**21**]. The balm is used to alleviate muscle pain, insect bites, colds and chest congestion (Singh *et al.*, 2014). Debella *et al.*, (2003) demonstrated pain relieving activities of Ethiopian *O. lamiifolium* aqueous and methanolic extracts in mice.

Antimicrobial activity has also been observed in extracts of selected *Ocimum* species. Antibacterial activity of *O. kilimandscharicum* (Saha *et al.*, 2013; Runyoro *et al*., 2010) and *O. lamiifolium* (Kifle *et al*., 2007; Damtie and Mekonnen, 2015) have been reported

against a number of Gram positive and Gram negative bacteria. Antiplasmodial activity was observed in *O. kilimandscharicum* (Runyoro *et al.,* 2010) and *O. lamiifolium* (Kefe *et al.*, 2016) against chloroquine resistant *Plasmodium falciparum* and *Plasmodium berghei* clones, respectively.

Extracts of *O. lamiifolium* (Hakkim *et al.*, 2008; Kifle *et al.*, 2007) and *O. kilimandscharicum* (Nanak *et al.,* 2011) showed good antioxidant activity hence can be used as preservatives in food and cosmetic industries (Dambolena *et al*., 2010). They can also be used in prevention of phototoxity and treatment of inflammation as demonstrated in beta carotene-linoleic acid bleaching (Hakkim *et al.*, 2008) and DPPH *in-vitro* assays (Kifle *et al.,* 2007), respectively. Antidiabetic activity of aqueous O. *lamiifolium* leaf extract was observed when therapeutic doses were administered intraperitoneally and orally on alloxan induced diabetic mice (Arika *et al*., 2016). However, these studies did not describe the chemical compositions of the *O. lamiifolium* extracts. Findings of scientific analyses of selected *Ocimum* species support the traditional medicinal use of the plant concoctions in treatment of various ailments (Verma *et al.*, 2011).

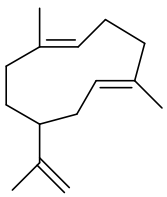
2.8.2 Pesticidal activity

Traditionally, *Ocimum* extracts are used as repellents against stored grain pests, particularly the weevil (Sarin *et al.*, 2013). Repellant toxicity and grain protectant activity was observed when eucalyptol, a major compound of *O. kenyense* essential oil was tested against stored grain pests (Bekele and Hassanali (2001). Eucalyptol [**22**] evoked a strong repellent action against *Sitophilus granarius* and *Sitophilus zeamais* while; moderately repelling *Tribolium castenum* and *Prostephanus trunctatus* (Golob, 2002). Essential oils of *O.*

kilimandscharicum and *O. americanum* were reported to exhibit insecticidal activities against maize weevil (*Sitophilus zeamais*) with *O. kilimandscharicum* showing higher insecticidal activity than *O. americanum* essential oil.

Camphor [**21**] and eucalyptol [**22**] are major components of *O. kilimandscharicum* and *O. americanum* essential oils, respectively (Mathu, 2015). Toxicity of *O. kenyense* and *O. kilimandscharicum* essential oils and selected blends of major constituents against *Sitophilus zeamais* and *Rhizopertha dominica* were also reported by Bekele and Hassanali (2001). In another study by Golob *et al.*, (1999), foliar essential oils of dried parts of *O. kilimandscharicum* exhibited 100% mortality in maize weevil (*S. zeamais*), lesser grain weevil (*R. dominica.*) and Angoumois grain moth (*Sititroga cerealella*).

Lwande *et al.*, (2017) patented a hive fumigant formulation meant to control honey bee pests such as mites, moths, ants and wasps among other bee hive pests. The fumigant contains *O. kilimandscharicum* essential oil extract as one of the components. The essential oil extract consists of compounds such as camphor [**21**], limonene [**23**], camphene [**24**], linalool [**27**], terpinen-4-ol [**34**], germacrene D [**47**] and geraniol [**17**] among others. The patented formulation is toxic to pests and non-toxic to bees hence it is suitable for bee hive fumigation.



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2.8.3 Mosquito repellence and larvicidal activity

In Western Kenya, local communities burn *Ocimum* plant species to keep away mosquitoes. To validate traditional use of *Ocimum* plants as mosquito repellents, scientific studies were conducted in semi field experimental huts in Rusinga Island. Direct burning and thermal expulsion of *O. kilimandscharicum* species leaves and seeds led to a two to fourfold reduction in human exposure to malaria vector (*A. gambiae*) as compared to volatile emission by fresh plants (Seyoum *et al.*, 2003). Essential oil of Ethiopian *O. lamiifolium* species showed larvicidal activity against third and early fourth instar stage larvae of *Anopholes arabiensis* and *Aedes aegypti* in simulated field experimental conditions (Marcus *et al.*, 2015) while Runyoro *et al.*, (2010) showed that *O. kilimandscharicum* and *O. lamiifolium* species essential oils exhibited larvicidal activity against *Culex quinqnefasciates*.

2.8.4 Bee attractant activity

Some traditional beekeepers use scented *Ocimum* species to spray molten old combs to attract honey bee swarms (Muli and Fraizer, 2011). The indigenous knowledge systems and Gachathi (1997), identifies *O. lamiifolium* (Gikuri), *O. kilimandscharicum* (Makuri) and *O. kenyense* (Macuki) as indigenous plant species traditionally used by beekeepers to lure honey bees into hives in Mt. Kenya region.

CHAPTER THREE

METHODOLOGY

3.1 Reagents, apparatus and standards

Solvents, adsorbent (Porapak Q) and chemical standards were obtained from Supleco (USA) and Sigma-Aldrich (Germany). All glassware was obtained from Telvian Agencies (Nairobi, Kenya) while dual choice Y-tube olfactometer was designed by MMUST and fabricated by Probe Engineering Ltd (Kisumu, Kenya). Reusable glassware was washed with hot water and soap, rinsed with cold water, acetone and finally with distilled water then dried at 110 ºC for one hour in an oven.

3.2 Site of the experiments

The study was carried out in Masinde Muliro University of Science and Technology (MMUST) chemistry laboratory as well as in a field site located at the Centre for African Medicinal and Nutritional Flora and Fauna (CAMNFF) also in MMUST, Kakamega County.

3.3 Collection of selected plant materials and experimental honey bees

Aerial parts of mature plants in flowering stage of *Ocimum kenyense*, *O. kilimandscharicum* and *O. lamiifolium* species were each collected from two different agro ecological zones in Kenya based on ethno-botanical knowledge. Sampling sites were randomly selected in parts of Nyeri, Kirinyaga, Laikipia, Nyandarua and Nakuru Counties where natural populations of *Ocimum* plant species exist and are traditionally used to lure honey bee colonies into uncolonized hives (Figure 3.1 and Appendix 1).

Figure 3.1: Map of sampling sites (Geo currents base map)

Selected plant samples were identified by Mr. Mutiso, a plant taxonomist and voucher specimen (Table 3.1) deposited at the department of Botany herbarium, University of Nairobi.

Table 3.1: Specimen vouchers of selected *Ocimum* plant species

Voucher Specimen No.	Name of plant	Region	Abbreviated name
AN001/2014	*O. kenyense*	Laikipia	OKE-LKP
AN002/2014	*O. kenyense*	Nyeri	OKE-NYR
AN003/2014	*O. kilimandscharicum*	Kirinyaga	OKI-KRN
AN004/2014	*O. kilimandscharicum*	Nyeri	OKI-NYR
AN005/2014	*O. lamiifolium*	Nyandarua	OLA-NYD
AN006/2014	*O. lamiifolium*	Nakuru	OLA-NKU

Adult honey bee (*Apis mellifera*) foragers used in behavioural experiments were obtained from a wild colony naturally nested in a cypress tree (*Cupressus lusitanica*) at the Centre for African Medicinal and Nutritional Flora and Fauna (CAMNFF) in Masinde Muliro University of Science and Technology (MMUST) (Plate 3.1).

Plate 3.1: Honey bee (*A. mellifera*) naturally nesting in a cavity of cypress tree in MMUST

Adult honey bee (*A. mellifera*) foragers used in the dual choice experiments were collected from a wild natural colony nesting in a cypress tree cavity. The bees were collected using an improvised bee catcher fabricated using a metal rod and a 1 litre plastic bottle with internal diameter of 10 cm and height of 25 cm Plate 3.2).

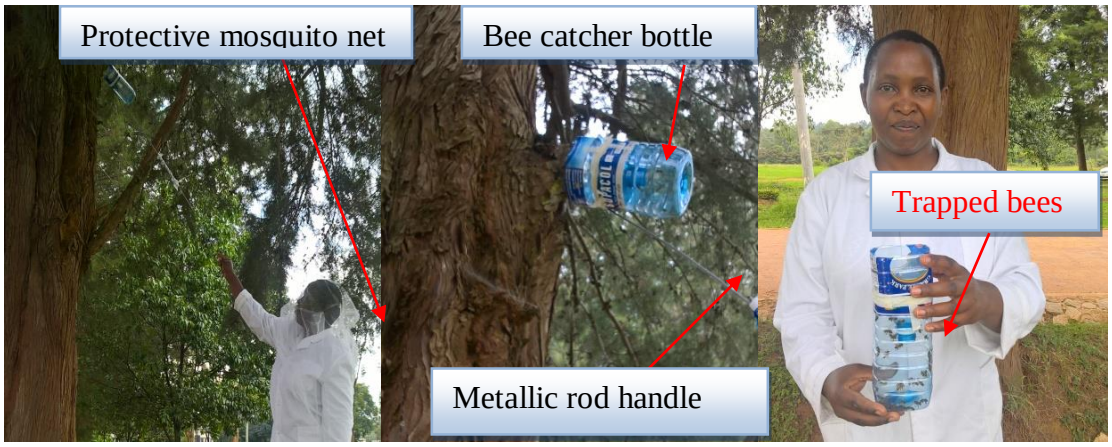


Plate 3.2: Collection of honey bees from a nesting site using an improvised bee catcher

3.4 Extraction of essential oil volatiles from selected *Ocimum* species

Fresh aerial parts (150g) of each species were steam distilled for 2 hours in an improvised distillation unit with a Dean and Stark modification (Plate 3.3). The essential oil distillates were dried over anhydrous sodium sulphate, packed in sealed glass vials and refrigerated at -10 ºC. Yields of essential oils of aerial parts of *O. kenyense, O. kilimandscharicum* and *O. lamiifolium* species were expressed in percentages of oil volume/ fresh weight of plant material used (% v/w).

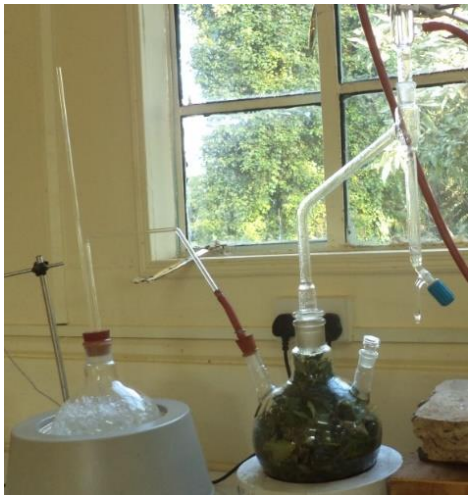


Plate 3.3: Extraction of essential oils from *Ocimum* species aerial parts by steam distillation

3.5 Air entrainment of fresh and smoldered volatiles of selected *Ocimum* species

Volatile collection traps (VCT) were prepared by packing glass column (60 mm x 4 mm) with Porapak-Q adsorbent (40 mg) mesh 80/100 (Supelco, USA). Glass wool was placed on both ends of the glass columns to hold the adsorbent in place (Plate 3.4). The packed VCTs were cleaned using dichloromethane for 12 hours in Soxhlet apparatus after which, they were conditioned for 24 hours in an oven at 50 ˚C. Porapak-Q VCTs were used to collect volatile organic compounds in both fresh and smoke emissions as described by Kozen (2013) and Weckerie *et al.*, (2011).

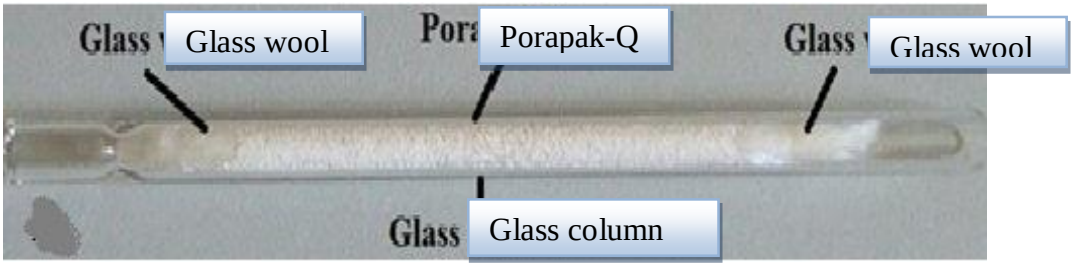


Plate 3.4: Porapak-Q Packed Volatile Collection Trap (VCT)

Clean air was pulled through an empty glass (1 litre) into a VCT to provide a control sample. Ten grams of fresh aerial parts of each selected *Ocimum* species were enclosed in clean 1 L glass bottle fitted with Porapak-Q trap and placed in a clean metallic box (15 cm × 15 cm × 20 cm) (Plate 3.5A). Headspace volatiles were collected in dynamic mode by pulling clean air through the plant material into the VCT using air entrainment kit for 20 minutes at a flow rate of 1L/Min as described by Babikova *et al*., (2013) and Dekker *et al*., (2011).

The aerial plant parts were air dried under shade for three days prior to collection of smoldered volatiles. Two medium sized pieces of glowing charcoal were placed in a two litre steel container with a tight fitting lid and clean air pulled into a VCT using an air entrainment kit to provide a control sample for the smoldered volatiles. Ten grams of plant materials of each selected *Ocimum* species were separately placed on glowing charcoal in oval steel container of 20 cm diameter and 10 cm length and allowed to smolder for two minutes as described by Dube *et al.* (2011). A VCT was fitted into the lid and head space volatiles collected in a dynamic mode by pulling clean air into the VCT using air entrainment kit at a flow rate of 1L/Min for 20 minutes (Plate 3. 5B)

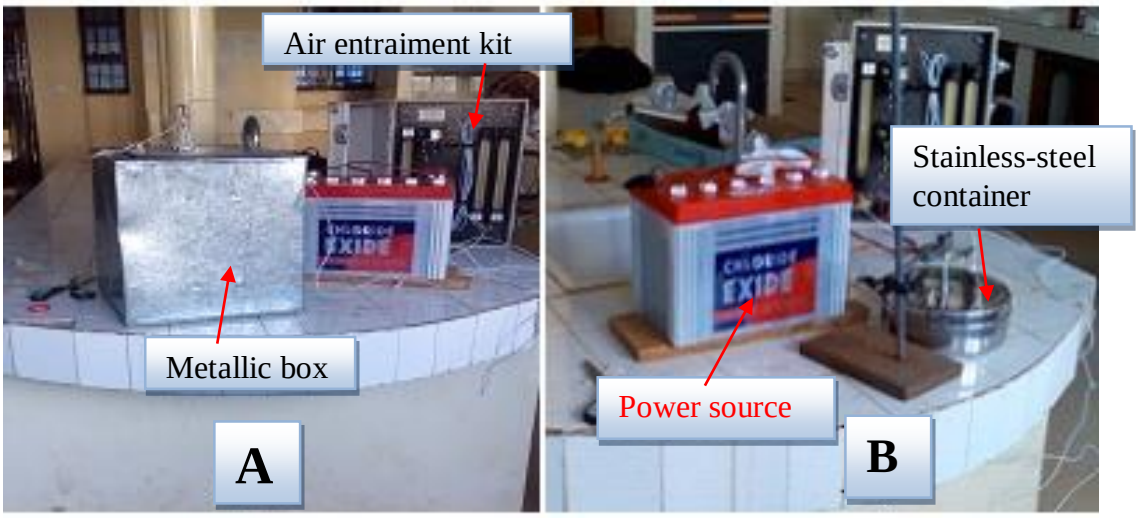


Plate 3.5: Air entrainment of fresh (A) and smoldered (B) volatiles

The VCT containing adsorbed *Ocimum* volatiles were sealed in a labeled glass tube to prevent contamination prior to chemical analyses. The traps containing freshly emitted, smoldered and control volatiles were eluted using 0.75 ml dichloromethane under ice. The eluted volatiles were transferred into borosilicate glass vials with a Teflon screw cap, labeled and refrigerated at -20˚C until required for use.

3.6 Determination of volatile chemical constituents of selected *Ocimum* species using GC-MS

The chemical constituents of *Ocimum* volatiles were determined using Gas Chromatography-Mass Spectrometry (GC-MS) analyses. GC-MS analyses were performed on HP GC 1800 II equipped with DB-5 MS column (30 m x 0.25 mm, 0.25 mm film thickness). Mass spectra were acquired on E1 mode (70 eV) in m/z range of 0-400 a.m.u with a scan time of 1.5 seconds. Carrier gas used was helium at flow rate of 1ml/min and split ratio 1:30. The injector temperature was 250 ºC; detector temperature was set at 270 ºC, while column temperature was linearly programmed at 40-240 ºC (at the rate of 5ºC/min).

The chemical constituents of selected *Ocimum* species volatiles were identified on the basis of their retention indices (RI) and comparison of mass spectra fragmentation patterns stored in MS library (NIST and Wiley database) as well as reference to literature (Adams, 2007; Babushok *et al*., 2011). Quantification of components was done by correlation of peak area percent obtained when a known amount of internal standard was added to each sample during analyses (Wang *et al*., 2017).

3.7 Determination of behavioural responses of bees to *Ocimum* species volatiles in olfactometric bioassay

To determine the attractiveness of essential oils, fresh and smoldered volatiles of *O. kenyense*, *O. kilimandscharicum* and *O. lamiifolium* to honey bee (*Apis mellifera scutellata*) workers, several dual choice olfactometric experiments were conducted. A Y-tube olfactometer with a body length of 100 cm and diameter of 20 cm and two arms each

measuring 30 cm in length and 15 cm in diameter, was used to conduct several dual choice experiments (Plate 3.6).

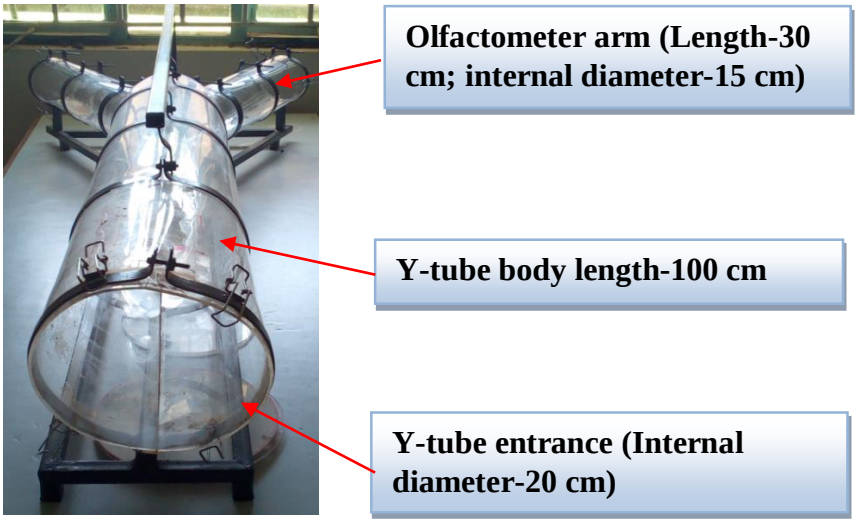


Plate 3.6: Y-tube olfactometer used in dual choice experiment

A passive flow system was used during the dual choice experiments where air was allowed to flow freely through the olfactometer so as to mimic the natural environment in which bees perceive plant scents. All experiments were conducted between 8.00 a.m. and 2.00 p.m. on sunny days when bee foraging activity was high (Doetterl *et al.*, 2014). The experimental set up for dual choice Y tube olfactometer bioassays is shown in Plate 3.7 below.

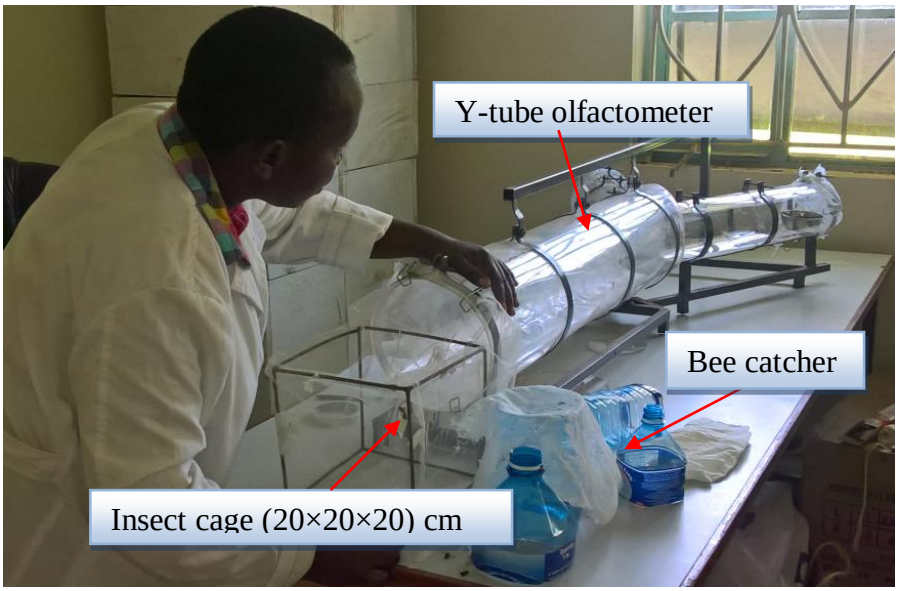


Plate 3.7: Dual choice Y-tube olfactometer experimental set up

In each test, bee foragers (n=10) were released from the insect cage at the entrance of Y tube and allowed to choose between odour and control arms. The bees number of bees in odour and control arms were counted and recorded while those that remained in the Y tube were excluded from the test result. The bees were released from the olfactometer after each test, olfactometer odour and control arms cleaned with distilled water and allowed to dry for ten minutes. Each test was replicated six times with odour and control arms being swapped to eliminate effects of test bees' bias towards one arm of the olfactometer. Mean values of bees attracted to odour and control arms were determined and used to determine bee attractant activity of various *Ocimum* species volatiles and their synthetic blends.

3.7.1 Determination of behavioural responses of bees to *Ocimum* species essential oil volatiles

To determine the attraction of honey bees to selected *Ocimum* species' essential oil volatiles, a small capillary tube was inserted into Teflon screw cap of a glass vial containing 100 µl of essential oil so as to dispense the odour in one arm of the olfactometer. An empty glass vial with a capillary tube inserted into the screw cap was placed in the other arm (control) (Plate 3.8). The number of bees in each arm of the olfactometer was counted and recorded in each replicate.

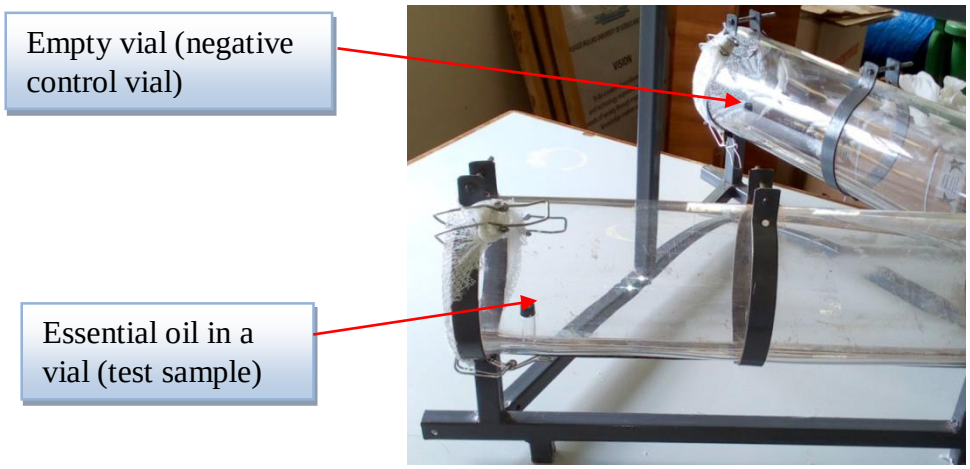


Plate 3.8: Experimental set up of dual choice bioassay of essential oil volatiles on the bees

3.7.2 Determination of behavioural responses of bees to *Ocimum* species fresh volatiles

Honey bee responses to fresh volatiles of selected *Ocimum* species aerial parts were determined in a dual choice Y tube olfactometer experiment according to Kogel *et al*., (1999) procedure. The aerial parts of *O. kenyense* (OKE), *O. kilimandscharicum* (OKI) and *O. lamiifolium* (OLA) were plucked from wild populations in their respective sampling sites, wrapped in moist tissue papers and placed in oven bags to reduce volatiles' loss

during transportation to the laboratory. The plant specimens were labeled as OKE-LKP, OKE-NYR, OKI-KRN, OKI-NYR, OLA-NYD and OLA-NKU (Table 3.1).

Ten grams of fresh plant material was wrapped in a white net and placed on one arm as an odour source while a clean stone wrapped in white net was placed in the other arm as a control. The shapes of wrapped stone and fresh plant material were made similar so as to eliminate visual bias on test bees (Plate 3.9). The number of bees in each arm of the olfactometer was counted and recorded in each replicate.

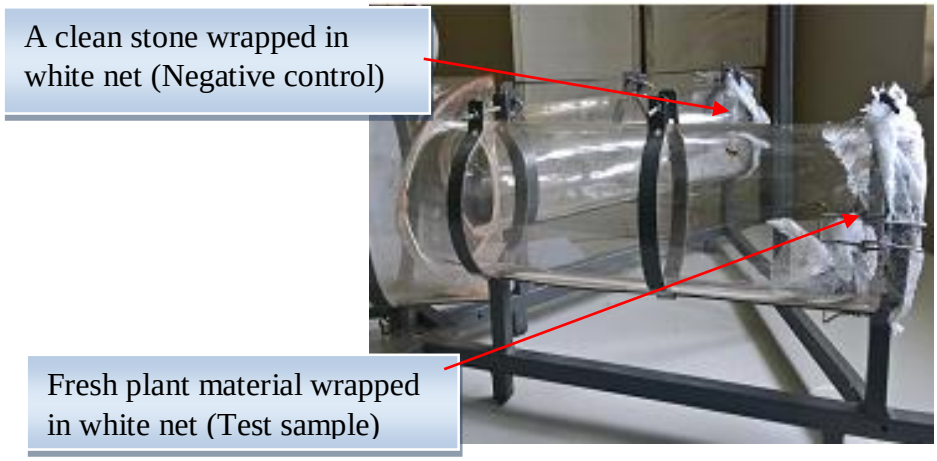


Plate 3.9: Experimental set up of dual choice bioassay of fresh volatiles

3.7.3 Determination of behavioural responses of bees to *Ocimum* species smoldered volatiles

To determine the attraction of honey bees to selected *Ocimum* species smoldered volatiles, aerial parts of selected *Ocimum* species were air dried under shade for three days after which, 10 g of plant material was smoldered on glowing charcoal for 5 minutes in a steel container. Smoldering plant material was placed in one arm of the olfactometer as an odour source. A similar empty steel container was placed on the other arm as a control (Plate

3.10). The number of bees in each arm of the olfactometer was counted and recorded in each replicate.

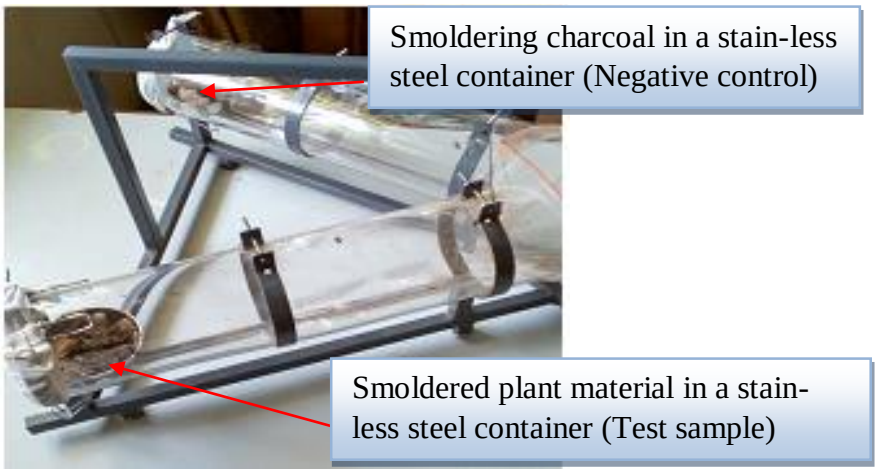


Plate 3.10: Experimental set up of dual choice bioassay of smoldered volatiles

3.7.4 Determination of behavioural responses of bees to *Ocimum* species volatiles' blends

Ocimum species volatiles found to be highly attractive to honey bees when tested against a negative control were tested for synergistic effect on bee attractant activity. Two component blends of fresh and smoldered volatiles of *Ocimum* species volatiles were separately prepared. Each 10 g blend consisted of 5 g of each of the two component *Ocimum* species plant materials. The rest of the protocol for fresh and smoldered volatile blends was conducted as described in sections 3.7.2 and 3.7.3, respectively. The number of bees in each arm of the olfactometer was counted and recorded in each replicate.

3.7.5 Determination of behavioural responses of bees to *Ocimum* species' volatiles and bee wax

The volatiles that displayed high bee attractant activity with reference to the negative control were tested against bee wax (positive control) which is a known attractant (Babarinde *et al.*, (2015). To determine honey bee responses to bee wax, 10 g of bee wax was wrapped in white net and placed in odour arm of the olfactometer while a similarly wrapped clean stone provided a negative control for the test. The number of bees in each arm of the olfactometer was counted and recorded in each replicate.

To determine honey bee responses to *Ocimum* species volatiles in presence of bee wax as a positive control, 10 g of fresh plant material and bee wax were separately wrapped in white net and placed in respective odour and control arms of the olfactometer. Similarly, grams of smoldered plant material were placed in a steel container in odour arm of the olfactometer while bee wax in another steel container was placed in control arm. The number of bees in each arm of the olfactometer was counted and recorded in each replicate.

To determine if there were synergism between *Ocimum* species' volatiles and bee wax, a blend of 5 g of the most active *Ocimum* species plant materials were blended with 5 g of bee wax and placed in the odour arm of olfactometer and a negative control was placed in the other arm. The experiment was also conducted with bee wax as a positive control. The rest of the protocol was conducted as described in sections 3.7.2 and 3.7.3. The number of bees in each arm of the olfactometer was counted and recorded in each replicate.

3.8 Determination of bee attractant activity of *Ocimum* species' volatiles in field bioassay

A completely randomized Latin square block (CRLSB) 8 × 8 experimental design was set to determine the swarm luring activity of olfactometric active volatiles in the field. To minimize bias, the bee hives positions were alternated after every 40 days throughout the experiment which lasted for one year to cater for dry and wet seasons (Appendix 2). The treated hives based on the most olfactometric active volatiles (1, 2, 3, 4, 6 and 8) and controls (5 and 7) were placed at eight sites at a distance of 50 metres from each other (Carroll, 2006).

Small Kenya Top Bar Hive (KTBH) measuring 45 cm x 36 cm x 22 cm with 9 bars were used for the field experiment whereby 10 g of dried flowering tops of smoldering *O. kilimandscharicum* (Kirinyaga), *O. kenyense* (Nyeri) and *O. kilimandscharicum* (Nyeri) were separately enclosed in three hives for 30 minutes. Similarly, interior parts of three hives were separately rubbed with 10 g of freshly plucked flowering tops of *O. kilimandscahricum* (Kirinyaga), *O. kenyense* (Nyeri) and *O. kilimandscharicum* (Nyeri) respectively (Plate 3.11). Bee wax (positive control) was applied on interior parts of one hive while another hive was left untreated to provide a negative control.

Plate 3.11: Exterior (A) and interior (B) part of beehive and treatment of smoldered (C) and fresh (D) *Ocimum* species volatiles

The treated hives were placed in a lockable metallic cage at a height of 2 M above the ground (Plate 3.12A). The hives were monitored daily for colonization and the amount of time in days taken for each hive to be colonized recorded. At the end of every experimental cycle, the occupied hives were moved to a bee house (Plate 3.12 B and C) to allow use of the sites in subsequent cycles.

Plate 3.12: An experimental hive in a lockable metallic stand (A), a bee house (B) and KTBH hives (C) inside the bee house

3.9 Determination of bee attractant activity of synthetic blends of *Ocimum* species' volatiles in olfactometric bioassay

Synthetic blends of major chemical constituents of the most attractive treatments in laboratory and field experiments were prepared in relative proportions as found in respective GC-MS profiles (Gikonyo *et al.*, 2003; Krug *et al*., 2018). Several minor chemical constituents of the most attractive volatiles previously reported as bee attractants were also included in the synthetic blends. The percentage compositions of synthetic blends were between 61.33-70.40% since some of the major chemical constituents that were unavailable were excluded from respective blends. A total of twenty-one chemical standards (purity>98%) were obtained from Sigma and Aldrich Company (Germany) and used to prepare six synthetic blends.

Relative concentrations of chemical components of each synthetic blend were calculated in weight per volume (µg/ml) based on percentage concentrations of internal standards used in GC-MS analysis of selected *Ocimum* species' fresh and smoldered volatiles. Calculation of concentration of chemical constituents of synthetic blends was done according to equation 1.

$$Concentration\ (\mu g/ml) = \frac{\%\ Concentration \times 50\ \mu g\ (Internal\ standard)}{\%\ Concentration\ of\ internal\ standard} \ldots\ldots\ldots \text{Equation 1}$$

Volumes of liquid chemical constituents were determined according to equation 2.

$$Volume\ (\mu l) = \frac{Mass\ (\mu g)}{Density\ (\mu g/ul)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 2}$$

Eight, ten and twelve component synthetic blends of fresh volatiles of *O. kenyense*-Nyeri (OKENF), *O. kilimandscharicum*-Nyeri (OKINF) and *O. kilimandscharicum*-Kirinyaga

(OKIKF) were prepared according to respective concentrations of chemical constituents as determined using equations 1 and 2. Similarly, six, eleven and ten component synthetic blends of smoldered volatiles of *O. kenyense*-Nyeri (OKENS), *O. kilimandscharicum*-Nyeri (OKINS) and *O. kilimandscharicum*-Kirinyaga (OKIKS) were also prepared according to the respective concentrations of chemical constituents as determined using equations 1 and 2.

3.9.1 Determination of behavioural responses of bees to synthetic blends

Each synthetic blend of selected chemical constituents based on their relative proportions in GC-MS profiles were tested for their bee attractant activity in a Y-tube olfactometer. A capillary tube of internal diameter 2 mm was inserted into a Teflon screw cap of a glass vial containing 100 µl of synthetic blend so as to dispense the odour in one arm of the olfactometer. An empty glass vial was placed in the other arm to provide a negative control for the test. The experiment was repeated using a mixture of 100 µl of synthetic blend and 100 µl of acetone in a vial with a capillary tube inserted into the screw cap as an odour source. A similar glass vial containing 100 µg of bee wax and 100 µl acetone formed a positive control for the test. Mean values of honey bees attracted to odour and control sources were determined.

3.9.2 Determination of behavioural responses of bees to various synthetic blends in subtraction bioassays

Subtraction bioassay was conducted so as to determine specific constituent(s) that contribute(s) significantly to bee attractant activity of the most active synthetic blends. Each subtraction bioassay started with subtraction of chemical constituents known to be

unattractive to bees from the literature (Huber, 2016; Chadzon and Whitmore, 2002). According to Byer (1992) chemical constituents whose subtraction from the full blend cause an increase in attractant activity are usually excluded from the test blends in subsequent bioassays.

Each chemical constituent of synthetic blends of smoldered volatiles of *O. kenyense*-Nyeri (OKE-NYR), *O. kilimandscharicum*-Kirinyaga (OKI-KRN) and *O. kilimandscharicum*-Nyeri (OKI-NYR) and was excluded at a time during subtraction bioassays. Seven, ten and twelve synthetic blends of OKE-NYR, OKI-KRN and OKI-NYR smoldered volatiles, respectively were tested for bee attractant activity in Y-tube olfactometer against negative control as described in section 3.9.1. Mean values of honey bees attracted to odour and control sources were determined.

3.9.3 Determination of behavioural responses of bees to synthetic blends of the most active chemical constituents

Ten chemical constituents of smoldered volatiles of *O. kilimandscharicum*-Kirinyaga, *O. kilimandscharicum*-Nyeri and *O. kenyense*-Nyeri species found to contribute significantly to attraction of honey bees in subtraction bioassays were identified and used to prepare three synthetic blends namely SBAC1, SBAC2 and SBAC3 comprising of ten, six and seven chemical constituents in varying ratios of 12:14:8:2:2:4:5:1:1, 12:14:8:2:2:4 and 12:14:8:2:2:4:3 and respectively. Each of the three synthetic blends was assayed against a negative control to determine responses of honey bees in dual choice Y-tube olfactometer as described in section 3.9.1. Mean values of honey bees attracted to odour and control sources were determined.

3.9.4 Determination of Minimum Efficative Concentrations of most active synthetic blend

In this experiment, acetone was used as a carrier solvent since it is known to be attractive and non-toxic to honey bees (Robinson *et al*., 2017). Four dilutions of 100 µg/µl (97 µg/µl SBAC1 + 3 µl acetone), 75 µg /µl (72.8 µg/µl SBAC1 + 2.2 µl acetone), 50 µg/µl (48.5 µg/µl SBAC1 + 1.5 µl acetone) and 25 µg/µl (24.2 µg/µl SBAC1 + 0.8 µl acetone were prepared. The four dilutions of SBAC1 blend were assayed against 3, 2.2, 1.5 and 0.8 µl of acetone, respectively to determine the minimum concentration that attracted 50% ($MEC_{50)}$ and 75% (MEC_{75}) of the test honey bees. Probit analysis was conducted in Excel and minimum concentrations of SBACI blend determined according this equation 3.

$y = 7.051x + -0.927$……Equation 3

where y=probit value and x=MEC.

3.10 Statistical data analysis

Percentage mean values of essential oil yields of *O. kilimandscharicum, O. kenyense* and *O. lamiifolium* species were determined using SAS and compared for significant differences based on species and agro ecological zone of origin. In each dual choice Y-tube olfactometer bioassay, means of honey bees attracted to odour and control source as well as means of time taken (days) for a hive to be occupied in a field bioassay per site, cycle and treatment were also determined using SAS (2000). In each case of analysis, the means were compared using their respective Least Square Differences (LSD) (t test) at $p<0.05$ so as to determine if they were significantly different. Samples of statistical analysis data sheets of laboratory and field bioassays are presented in appendices 3, 4, 5, 6 and 7.

CHAPTER FOUR

RESULTS

4.1 Yields of essential oil volatiles of selected *Ocimum* species

Yields of essential oil of aerial parts of *O. kenyense, O. kilimandscharicum* and *O. lamiifolium* species were expressed in mean percentages of volume per fresh weight of plant material used (% v/w). Means of essential oil yield for each species and agro ecological zone were determined using SAS and separated using t test (LSD) at $p<0.05$ (Table 4.1 and Appendix 3).

Table 4.1: Percentage mean yield of essential oil yields of selected *Ocimum* species growing in various agro ecological zones

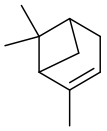
Species	Yield per species (v/w)	Agro ecological zone	% Yield
O. kenyense	0.397 ± 0.005^{b}	Laikipia	0.306 ± 0.01^{bc}
		Nyeri	0.360 ± 0.01^{b}
O. kilimandscharicum	0.494 ± 0.005^{a}	Kirinyaga	0.346 ± 0.01^{b}
		Nyeri	0.554 ± 0.01^{a}
O. lamiifolium	0.325 ± 0.005^{c}	Nyandarua	0.230 ± 0.01^{d}
		Nakuru	0.294 ± 0.01^{c}

NB: Means in a column followed by different letters are significantly different at $\alpha=0.05$

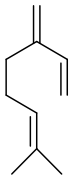
Mean percentage yields of essential oils of O. *kilimandscharicum, O. kenyense* and *O. lamiifolium* species were significantly different ($p<0.05$) with values of 0.49%, 0.40% and 0.33% ($p<0.05$), respectively. Essential oil yields of each species varied significantly with agro ecological zone of origin. Nyeri and Nyandarua agro ecological zones recorded the highest and lowest yields of 0.55% and 0.23%, respectively.

4.2 Chemical composition of selected *Ocimum* species' essential oil volatiles

GC-MS analyses led to identification of a total of twenty-nine, thirty-two and nineteen chemical components in *O. kilimandscharicum, O. lamiifolium* and *O. kenyense* species' essential oils, respectively. α-Pinene [**48**] (0.67-3.06%), β-myrcene [**49**] (0.85-6.60%), eucalyptol [**22**] (0.41-24.6%) and (*E*)-β-caryophyllene [**35**] (0.86-5.48%) were identified in essential oils of all investigated *Ocimum* species as some of the major constituents among others (Table 4.2, 4.3 and 4.4).



(48)



(49)

4.2.1 Chemical composition of *O. kenyense* essential oil volatiles

A total of nineteen chemical constituents were identified in essential oil volatiles of *O. kenyense* species from both Laikipia and Nyeri Counties (Table 4.2).

Table 4.2: Chemical constituents of *O. kenyense* essential oil volatiles

GC Peak	RI	Identity of the compound	Amount in %	
			Laikipia	**Nyeri**
1	840	Ethyl-methylbutyrate	-	0.91
2	849	Ethyl isovalerate	1.50	4.49
3	932	α-Pinene	3.06	1.16
4	974	β-Pinene	-	3.22
5	988	β-Myrcene	6.60	1.02
6	990	2-Octanal	0.45	-
7	1022	*o*-Cymene	2.12	-
8	1024	Limonene	0.64	-
9	1026	Eucalyptol	24.61	20.24
10	1054	ɤ-Terpinene	0.71	0.61
11	1129	β-Terpineol	1.13	0.94
12	1194	(*Z*)-Piperitol	0.99	0.61
13	1195	Estragole	35.06	22.19
14	1253	Chavicol	6.78	6.74
15	1417	(*E*)-β-Caryophyllene	0.86	3.38
16	1452	α-Humulene	2.77	10.37
17	1505	β-Bisabolene	4.23	14.18
18	1587	Geranyl isovalerate	-	1.19
19	1672	β-Bisabolol	2.48	-
		Monoterpenoids	**37.73**	**27.80**
		Sesquiterpenoids	**10.34**	**27.93**
		Benzenoids	**43.96**	**6.59**
		Non-terpenoids	**1.50**	**28.93**

Sixteen compounds were identified in essential oil volatiles of *Ocimum kenyense* from Laikipia County (OKE-LKP). Several major compounds such as β-myrcene [**49**] (peak 5) (6.60%), eucalyptol [**22**] (peak 9) (24.61%), estragole [**37**] (peak 13) (35.06%) and chavicol [**50**] (peak 14) (6.78%) characterized OKE-LKP essential oil volatiles. Minor chemical constituents such as α-pinene [**48**] (peak 3) (3.06%), α-humulene [**51**] (peak 16) (2.77%), β-bisabolene [**52**] (peak 17) (4.23%) and β-bisabolol [**53**] (peak 19) (2.48%) among others, were also identified in *O. kenyense* (LKP) essential oil (Figure 4.1).

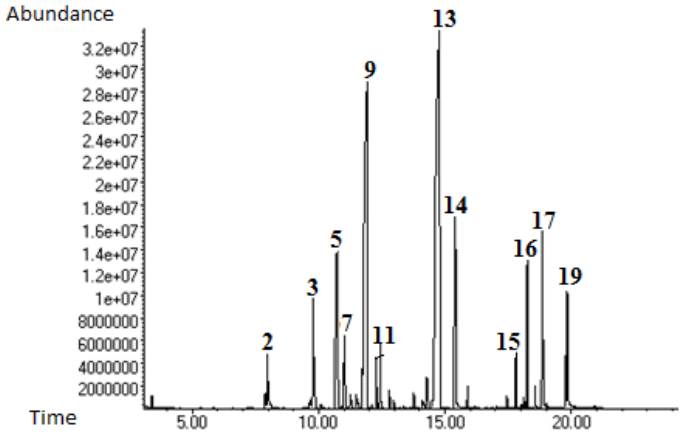


Figure 4.1: Total ion chromatogram of essential oil of *O. kenyense* (Laikipia County)

Eighteen chemical compounds were reported in *O. kenyense* essential oil volatiles from Nyeri County (OKE-NYR). The essential oil of OKE-NYR was characterized by five major constituents namely eucalyptol [**22**] (peak 9) (20.24%), estragole [**37**] (peak 13) (22.19%), chavicol [**50**] (peak 14) (6.74%), α-humulene [**51**] (peak 16) (10.37%) and β-bisabolene [**52**] (peak 17) (14.18%). Ethyl isovalerate [**54**] (peak 2) (4.49%), β-pinene [**55**] (peak 4) (3.22%), (*E*)-β-caryophyllene [**35**] (peak 15) (3.38%) and geranyl isovalerate [**56**] (peak 18) (1.19%) among others were also reported as minor constituents of the essential oil (Figure 4.2).

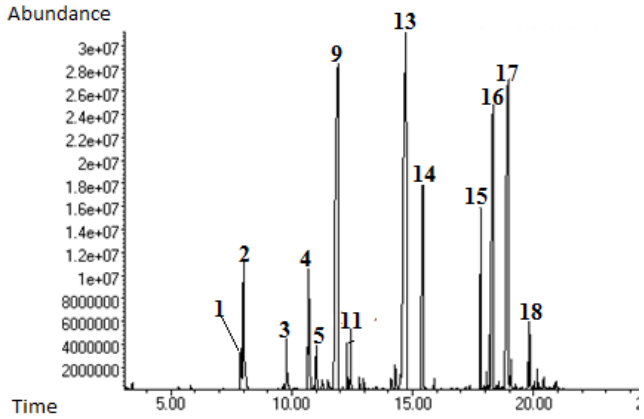


Figure 4.2: Total ion chromatogram of essential oil of *O. kenyense* (Nyeri County)

Monoterpenoid (27.8-37.7%), sesquiterpenoid (10.3-27.9%), benzenoid (6.6-43.9%) and non-terpenoid (1.5-28.9%) classes of chemical constituents were present in essential oil volatiles of *O. kenyense* species from both Laikipia (OKE-LKP) and Nyeri (OKE-NYR). Eucalyptol [**22**] (20.2-24.6%), estragole [**37**] (22.2-35.1%) and chavicol [**50**] (6.74-6.78%) were the key constituents of both OKE-LKP and OKE-NYR essential oil volatiles. However, OKE-LKP essential oil had a higher content of estragole [**37**] (35.1%) and eucalyptol [**22**] (24.6%) as compared to OKE-NYR essential oil respective contents of 20.2% and 22.2%. On the other hand, α-humulene [**51**] (2.77-10.37%) and β-bisabolene [**52**] (4.23-14.18%) occurred as a major and minor compound of respective OKE-LKP and OKE-NYR essential oil volatiles. β-Bisabolol [**53**] was a unique marker of OKE-LKP essential oils.

4.2.2 Chemical composition of *O. kilimandscharicum* essential oil volatiles

A total of twenty-nine chemical constituents were identified in essential oil volatiles of *O. kilimandscharicum* species from both Kirinyaga and Nyeri Counties (Table 4.3).

Table 4.3: Chemical constituents of *O. kilimandscharicum* essential oil volatiles

GC Peak	RI	Identity of the compound	Amount in %	
			Kirinyaga	**Nyeri**
1	932	α-Pinene	0.67	1.66
2	946	Camphene	3.35	5.44
3	974	β-Pinene	-	1.99
4	988	β-Myrcene	5.14	1.84
5	1002	α-Phellandrene	0.56	0.61
6	1024	Limonene	5.55	4.25
7	1026	Eucalyptol	3.79	12.29
8	1032	(*Z*)-β-Ocimene	1.24	1.67
9	1044	(*E*)-β-Ocimene	4.43	-
10	1054	ɤ-Terpinene	0.72	1.06
11	1083	Fenchone	-	1.47
12	1086	Terpinolene	2.97	4.56
13	1095	Linalool	1.32	4.17
14	1118	Exo-fenchol	-	1.18
15	1140	Neo-allo-ocimene	1.21	-
16	1141	Camphor	21.15	27.36
17	1165	Borneol	0.87	2.22
18	1174	Terpinen-4-ol	-	1.55
19	1186	α-Terpineol	0.82	1.76
20	1208	(*E*)-2-Octenyl acetate	-	1.67
21	1227	Nerol	1.76	-
22	1235	Neral	1.12	-
23	1249	Geraniol	14.50	-
24	1254	Geranial	1.81	-
25	1379	Geranyl acetate	4.24	-
26	1417	(*E*)-β-Caryophyllene	5.48	2.99
27	1484	Germacrene-D	1.36	2.62
28	1504	(*E*,*E*)-α-Farnesene	-	1.96
29	1523	Eugenol acetate	1.58	1.25
		Monoterpenoids	**72.98**	**75.08**
		Sesquiterpenoids	**6.84**	**7.57**
		Benzenoids	**5.82**	**2.92**

GC-MS analysis of *O. kilimandscharicum* species essential oil volatiles from Kirinyaga County (OKI-KRN) led to identification of twenty-three compounds. Out of these, five major compounds such as β-myrcene [**49**] (peak 4) (5.14%), limonene [**23**] (peak 6) (5.55%), camphor [**21**] (peak 16) (21.15%), geraniol [**17**] (peak 23) (14.5%) and (*E*)-β-

caryophyllene [**35**] (peak 26) (5.48%) characterized OKI-KRN essential oil. Camphene [**24**] (peak 2) (3.35%), eucalyptol [**22**] (peak 7) (3.79%), (*E*)-β-ocimene [**20**] (peak 9) (4.43%), nerol [**19**] (peak 21) (1.76%), neral [**18**] (peak 22) (1.12%), geranial [**16**] (peak 24) (1.81%) and geranyl acetate [**57**] (peak 25) (4.24%) among others, occurred as the essential oil volatiles' minor constituents (Figure 4.3).

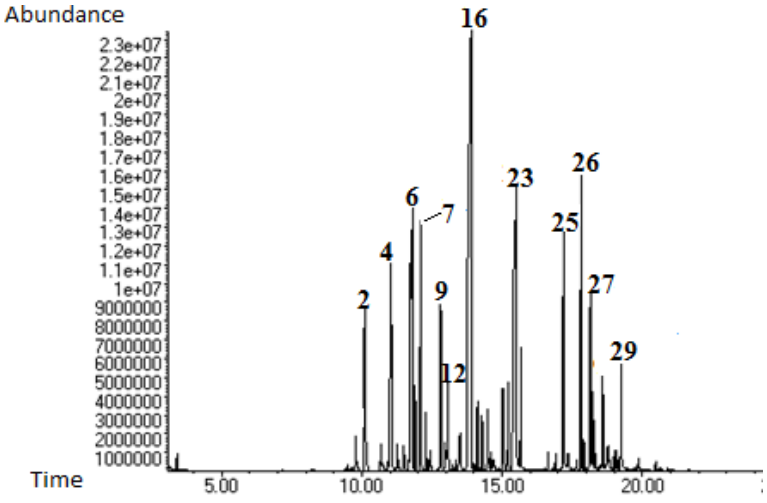


Figure 4.3: Total ion chromatogram of essential oil of *O. kilimandscharicum* (Kirinyaga County)

A total of twenty-two chemical compounds were identified in essential oil volatiles of *O. kilimandscharicum* species from Nyeri County (OKI-NYR). Three major compounds namely camphene [**24**] (peak 2) (5.44%), eucalyptol [**22**] (peak 7) (12.29%) and camphor [**21**] (peak 16) (27.36%) characterized essential oil volatiles of OKI-NYR. Limonene [**23**] (peak 6) (4.25%), terpinolene [**58**] (peak 12) (4.56%), linalool [**27**] (peak 13) (4.17%), borneol [**26**] (peak 17) (2.22%), (*E*)-β-caryophyllene [**35**] (peak 26) (2.99%) and germacrene-D **[47]** (peak 27) (2.62%) among others, were also identified as minor compounds of the essential oil volatiles (Figure 4.4).

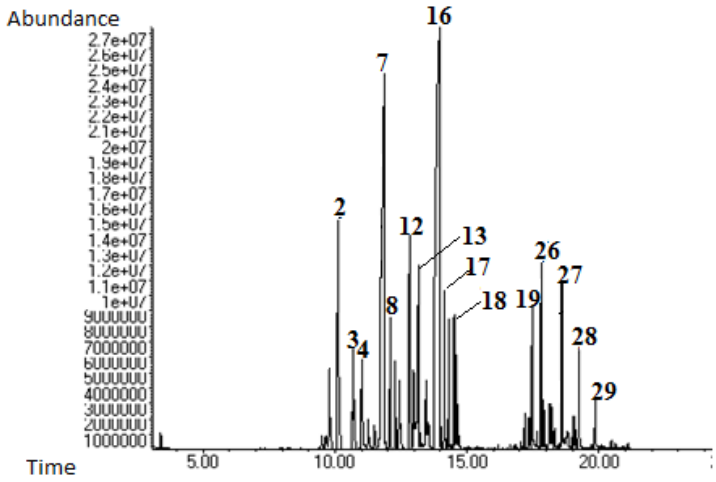
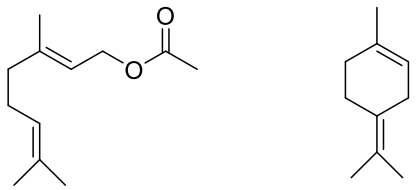


Figure 4.4: Total ion chromatogram of essential oil of *O. kilimandscharicum* (Nyeri County)



(57) (58)

Three major classes of compounds namely; monoterpenoid (72.9-75.1%), sesquiterpenoid (6.8-7.7%) and benzenoid (2.9-5.8%) were identified in essential oil volatiles of O. *kilimandscharicum* species from both Kirinyaga (OKI-KRN) and Nyeri (OKI-NYR). Camphor [**21**] (21.2-27.4%) was reported as a major compound of essential oil volatiles of both OKI-KRN and OKI-NYR. Geranial [**16**] (1.81%) and neral [**18**] (1.12%) were unique markers of *O. kilimandscharicum* (OKI-KRN) essential oil volatiles.

4.2.3 Chemical composition of *O. lamiifolium* essential oil volatiles

A total of thirty-two chemical constituents were identified in essential oil volatiles of *O. lamiifolium* species from both Nyandarua and Nakuru Counties (Table 4.4).

Table 4.4: Chemical constituents of *O. lamiifolium* essential oil volatiles

GC Peak	RI	Identity of the compound	Amount in %	
			Nyandarua	**Nakuru**
1	932	α-Pinene	1.49	1.23
2	953	Thuja-2,4-diene	1.30	0.98
3	980	1-Octen-3-ol	0.78	1.06
4	988	β-Myrcene	0.98	0.85
5	1002	α-Phellandrene	13.01	12.36
6	1008	3-Carene	0.78	0.67
7	1022	*o*-Cymene	2.42	2.10
8	1026	Eucalyptol	0.43	0.41
9	1030	2-Ethyl hexan-1-ol	6.42	-
10	1032	(*Z*)-β-Ocimene	4.98	4.70
11	1060	(*E*)-2-Octen-1-ol	-	5.10
12	1208	(*E*)-2-Octenyl acetate	5.13	7.53
13	1315	(*E*)-3-Hexenyl tiglate	0.95	0.73
14	1370	Carvacrol acetate	4.82	3.96
15	1387	β-Bourbonene	0.67	1.61
16	1387	β-Cubebene	1.24	-
17	1391	β-Elemene	-	1.31
18	1409	α-Gurjunene	3.71	-
19	1417	(*E*)-β-Caryophyllene	1.68	5.29
20	1452	α-Humulene	1.05	2.04
21	1454	(*E*)-β-Farnesene	-	2.29
22	1484	Germacrene-D	1.01	1.61
23	1492	(*Z*)-β-Guaiene	2.70	2.13
24	1493	Epi-cubebol	3.17	7.88
25	1503	Germacrene-A	7.16	6.01
26	1513	γ-Cadinene	-	2.02
27	1522	δ-Cadinene	1.85	1.03
28	1537	α-Cadinene	5.23	1.55
29	1574	Germacrene-D-4-ol	1.88	-
30	1602	Ledol	1.01	-
31	1626	Epi-α-cadinol	2.81	-
32	1652	δ-Cadinol	1.04	1.11
		Monoterpenoids	**23.12**	**21.20**
		Sesquiterpenoids	**36.21**	**35.88**
		Benzenoids	**12.19**	**12.22**
		Non-terpenoids	**8.18**	**8.16**

Twenty-eight chemical constituents were reported in *O. lamiifolium* species' essential oil from Nyandarua County (OLA-NYD). Five major compounds such as α-phellandrene [**31**]

(peak 5) (13.01%), 2-ethylhexan-1-ol [**59**] (peak 12) (5.13%), (*E*)-2-octenyl acetate [**60**] (peak 9) (6.42%) and germacrene-A [**61**] (peak 25) (7.16%) characterized OLA-NYD essential oil volatiles. Minor chemical constituents such as *o*-cymene [**29**] (peak 7) (2.42%), (*Z*)-β-ocimene [**33**] (peak 10) (4.98%), carvacrol acetate [**62**] (peak 14) (4.82%), α-gurjunene [**63**] (peak 18) (3.71%) and epi-cubebol [**64**] (peak 24) (3.17%) among others, were also reported in OLA-NYD essential oil volatiles (Figure 4.5).

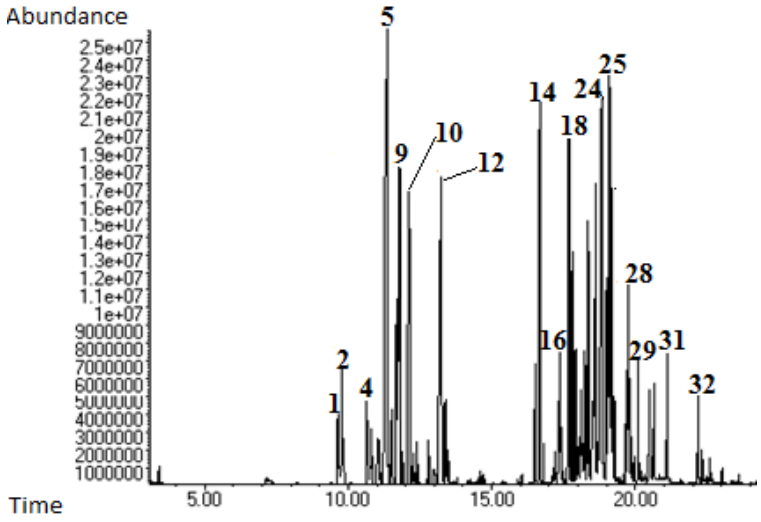


Figure 4.5: Total ion chromatogram of essential oil of *O. lamiifolium* (Nyandarua County)

Twenty-six chemical compounds were identified in essential oil of *O. lamiifolium* species from Nakuru County. Six major compounds namely α-phellandrene [**31**] (peak 5) (12.36%), (*E*)-2-octen-1-ol [**65**] (peak 11) (5.10%), (*E*)-2-octenyl acetate [**60**] (peak 12) (7.53%), (*E*)-β-caryophyllene [**34**] (peak 19) (5.29%), epi-cubebol [**63**] (peak 24) (7.88%) and germacrene-A [**61**] (peak 25) (6.01%) were reported in OLA-NKU essential oil volatiles. Minor compounds such as *o*-cymene [**29**] (peak 7) (2.10%), (*Z*)-β-ocimene [**33**] (peak 10) (4.70%) and carvacrol acetate [**62**] (peak 14) (3.96%) among others, were reported in OLA-NKU essential oil volatiles (Figure 4.6).

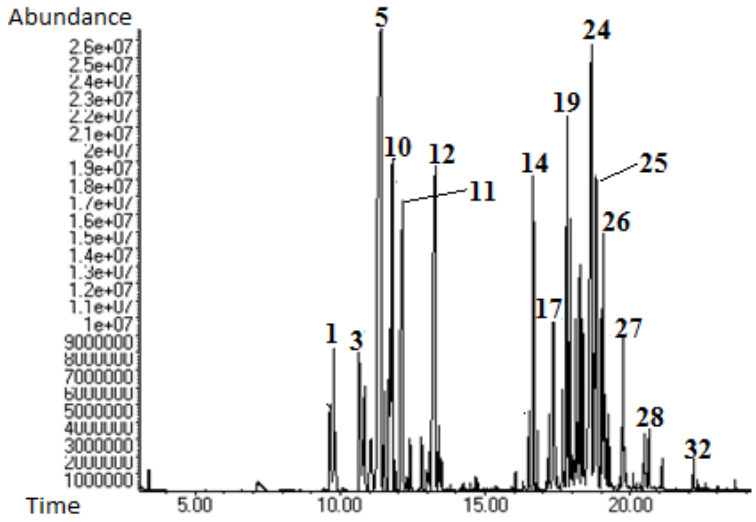
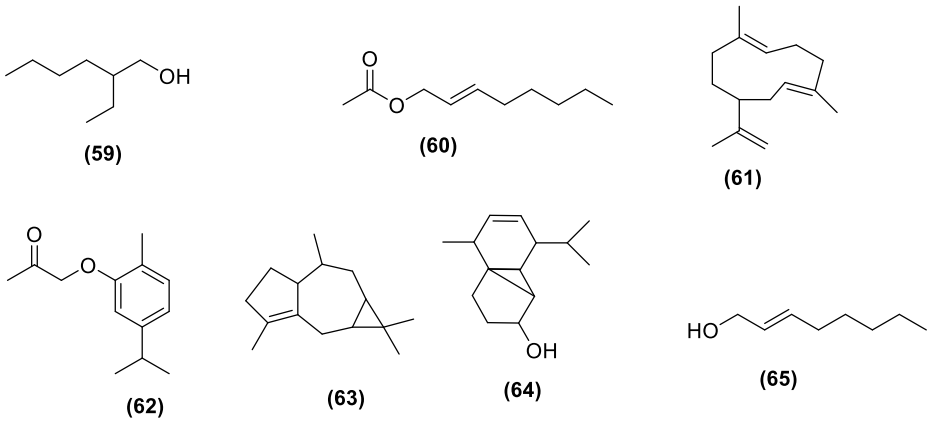


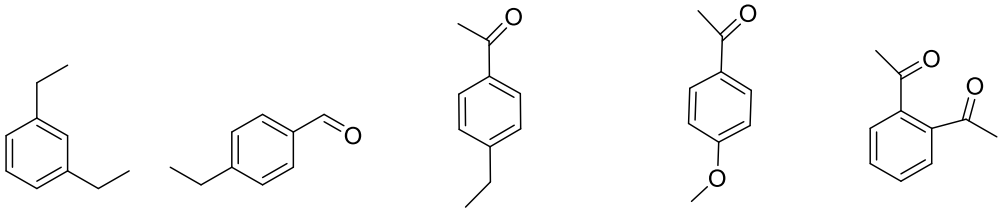
Figure 4.6: Total ion chromatogram of essential oil of *O. lamiifolium* (Nakuru County)

Essential oil volatiles of *O. lamiifolium* species from both Nyandarua (OLA-NYD) and Nakuru (OLA-NKU) were characterized by monoterpenoid (49.1-56.1%), sesquiterpenoid (10.2-12.3%) and benzenoid (16.7-25.7%) classes of compounds. α-Phellandrene [**31**] (12.4-13.0%), germacrene A [**61**] (6.0-7.2%), (*E*)-2-octenyl acetate [**60**] (5.1-7.5%) and epi-cubebol [**63**] (3.17-7.88%) were identified as major constituents of both OLA-NYD and OLA-NKU essential oils. Epi-α-cadinol [**64**] (2.81%) was a unique marker of OLA-NYD essential oil while γ-cadinene (2.02%) [**45**] was a unique marker of OLA-NKU essential oil.



4.3 Chemical composition of fresh volatiles of selected *Ocimum* species

GC-MS analyses of fresh volatiles of selected *Ocimum* species led to identification of a total of thirty, twenty-nine and sixteen chemical constituents in fresh volatiles of all investigated *Ocimum* species. Compounds such as 1,3-diethylbenzene [**66**] (1.06-2.31%), 4-ethylbenzaldehyde [**67**] (0.87-2.10%), *p*-ethylacetophenone [**68**] (3.85-11.31%), *p*-methoxyacetophenone [**69**] (3.84-11.68%), (*E*)-β-caryophyllene [**35**] (0.72-4.11%) and 1,2-diacetylbenzene [**70**] (1.11-2.10%) were identified in fresh volatiles of all investigated *Ocimum* species in varying concentrations (Table 4.2, 4.3 and 4.4). Control and blank chromatograms for fresh volatiles are presented in Appendix 8 (A and C).



(66) **(67)** **(68)** **(69)** **(70)**

4.3.1 Chemical composition of *O. kenyense* fresh volatiles

A total of sixteen chemical constituents were identified in fresh volatiles of *O. kenyense* species from both Laikipia and Nyeri Counties (Table 4.5).

Table 4.5: Chemical constituents of *O. kenyense* fresh volatiles

GC Peak	RI	Identity of the compound	Amount in %	
			Laikipia	**Nyeri**
1	849	Ethyl isovalerate	7.80	7.62
2	974	β-pinene	13.00	13.30
3	1026	Eucalyptol	8.80	6.10
4	1028	*p*-cymene	5.83	5.09
5	1030	*m*-cymene	5.38	5.00
6	1055	1,3-Diethyl benzene	2.19	2.31
7	1163	4-ethylbenzaldehyde	2.05	2.10
8	1174	2-ethylbenzaldehyde	0.82	0.76
9	1195	Estragole	1.15	1.05
10	1273	*p*-ethylacetophenone	9.65	11.31
11	1290	*p*-methoxyacetophenone	8.74	11.68
12	1417	(*E*)-β-Caryophyllene	1.20	1.26
13	1435	1,2-Diacetylbenzene	1.99	2.10
14	1451	1,4-Diacetylbenzene	1.55	2.09
15	1452	α-Humulene	-	4.93
16	1505	β-Bisabolene	2.25	6.10
		Monoterpenoids	**21.80**	**19.40**
		Sesquiterpenoids	**3.45**	**12.29**
		Benzenoids	**39.35**	**43.49**
		Non-Terpenoid	**7.80**	**7.62**

Fifteen compounds were identified in fresh volatiles of *O. kenyense* species from Laikipia County (OKE-LKP). The volatiles were characterized by seven major compounds namely ethyl isovalerate [**54**] (peak 1) (7.80%), β-pinene [**48**] (peak 2) (13.0%), eucalyptol [**22**] (peak 3) (8.80%), *o*-cymene [**29**] (peak 4) (5.83%), *m*-cymene [**71**] (peak 5) (5.38%), *p*-ethylacetophenone [**68**] (peak 10) (9.65%) and *p*-methoxyacetophenone [**69**] (peak 11) (8.74%). Minor chemical compounds such as β-bisabolene [**52**] (peak 16) (2.25%) and 1,3 diethylbenzene [**66**] (peak 6) (2.19%) among others, were also present in fresh volatiles of OKE-LKP (Figure 4.7).

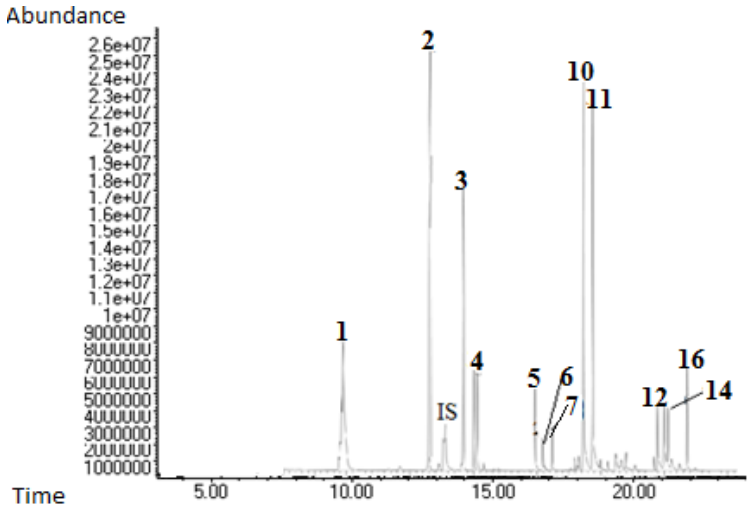


Figure 4.7: Total ion chromatogram of fresh volatiles of *O. kenyense* (Laikipia County)

Sixteen chemical compounds were reported in fresh volatiles of *O. kenyense* species from Nyeri County (OKE-NYR). The fresh volatiles were characterized by eight major compounds namely ethyl isovalerate [**54**] (peak 1) (7.62%), β-pinene [**55**] (peak 2) (13.34%), eucalyptol [**22**] (peak 3) (6.10%), *o*-cymene [**29**] (peak 4) (5.09%), *m*-cymene [**71**] (peak 5) (5.00%), *p*-ethylacetophenone [**68**] (peak 10) (11.31%), *p*-methoxyacetophenone [**69**] (peak 11) (11.68%) and β-bisabolene [**52**] (peak 16) (6.10%). Minor compounds such as α-humulene [**51**] (peak 15) (4.93%) and 1,3 diethylbenzene [**66**] (peak 6) (2.31%) among others, were also present in OKE-NYR fresh volatiles (Figure 4.8).

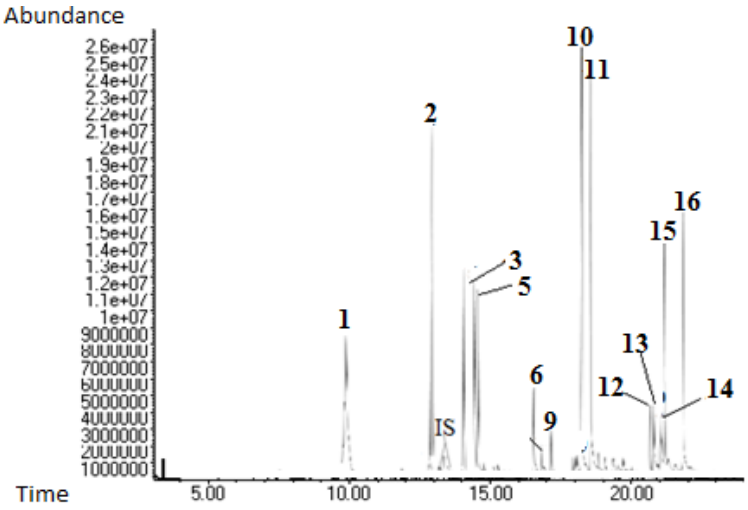
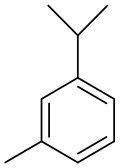


Figure 4.8: Total ion chromatogram of fresh volatiles of *O. kenyense* (Nyeri County)



(71)

Monoterpenoid (19.4-21.8%), benzenoid (39.4-43.5%), sesquiterpenoid (3.5-12.3%) and non-terpenoid (7.6-7.8%) dominated the fresh volatiles of *O. kenyense* species from both Laikipia (OKE-LKP) and Nyeri (OKE-NYR). Ethyl isovalerate [**54**] (7.6-7.8%), β-pinene [**55**] (13.0-13.3%), *p*-ethylacetophenone [**68**] (9.7-11.3%) and *p*-methoxyacetophenone [**69**] (8.7-11.6%) were identified as major constituents of OKE-LKP and OKE-NYR fresh volatiles. β-Bisabolene [**52**] (2.3-6.1%) occurred as a major and a minor constituent of respective fresh volatiles of OKE-NYR and OKE-LKP.

4.3.2 Chemical composition of *O. kilimandscharicum* fresh volatiles

A total of thirty chemical constituents were identified in fresh volatiles of *O. kilimandscharicum* species from both Kirinyaga and Nyeri Counties (Table 4.6).

Table 4.6: Chemical constituents of *O. kilimandscharicum* fresh volatiles

GC Peak	RI	Identity of the compound	Amount in %	
			Kirinyaga	**Nyeri**
1	932	α-pinene	2.08	1.73
2	946	Camphene	6.11	0.63
3	974	β-pinene	2.03	4.73
4	1022	*o*-cymene	5.30	5.80
5	1024	Limonene	9.94	-
6	1026	Eucalyptol	5.19	19.93
7	1040	1,4-Diethylbenzene	-	1.50
8	1044	(*E*)-β-ocimene	3.63	-
10	1074	Sabinene hydrate	0.81	1.01
11	1083	Fenchone	-	1.43
12	1093	6-Camphenone	3.46	-
13	1095	Linalool	-	19.20
14	1141	Camphor	19.40	1.42
15	1163	4-ethylbenzaldehyde	0.87	0.83
16	1168	Endo-borneol	0.53	-
17	1195	Estragole	-	3.02
18	1249	Geraniol	3.68	-
19	1273	*p*-ethylacetophenone	3.85	3.95
20	1290	*p*-methoxyacetophenone	3.88	3.84
21	1304	Lavandulyl acetate	1.08	-
22	1350	Eugenol	-	3.85
23	1351	α-Cubebene	-	1.00
24	1409	α-Gurjunene	-	0.53
25	1417	(*E*)-β-Caryophyllene	4.11	3.37
26	1435	1,2-Diacetylbenzene	1.60	1.86
27	1454	(*E*)-β-Farnesene	2.37	2.33
28	1472	γ-Muurolene	0.92	0.81
29	1484	Germacrene-D	1.35	2.61
30	1503	Germacrene-A	0.67	0.65
		Monoterpenoids	**56.05**	**49.07**
		Sesquiterpenoids	**10.23**	**12.31**
		Benzenoids	**16.68**	**25.71**
		Non-terpenoids	**1.08**	**-**

Twenty-three compounds were reported in fresh volatiles of *O. kilimandscharicum* species from Kirinyaga County (OKI-KRN). The fresh volatiles of OKI-KRN were characterized by five major compounds namely camphene [**24**] (peak 2) (6.11%), *p*-cymene [**41**] (peak 4) (5.30%), limonene [**23**] (peak 5) (9.94%), eucalyptol [**22**] (peak 6) (5.19%) and camphor [**21**] (peak 14) (19.47%). Minor compounds such as geraniol [**17**] (peak 18) (3.68%), (*E*)-β-ocimene [**20**] (peak 8) (3.63%), (*E*)-β-caryophyllene [**35**] (peak 25) (4.11%), *p*-ethylacetophenone [**68**] (peak 19) (3.85%), *p*-methoxyacetophenone [**69**] (peak 20) (3.88%) and 6-camphenone [**72**] (peak 12) (3.46%) among others, were also present in fresh volatiles of OKI-KRN (Figure 4.9).

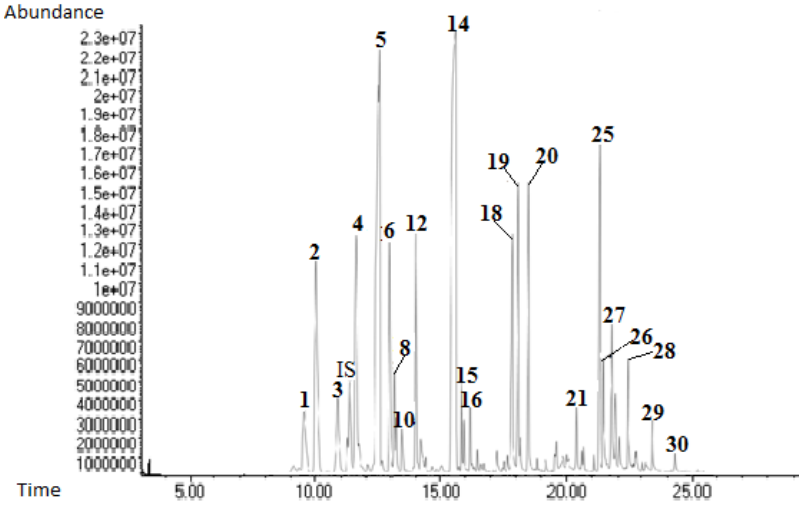


Figure 4.9: Total ion chromatogram of fresh volatiles of *O. kilimandscharicum* (Kirinyaga County)

Twenty-four chemical constituents were identified in fresh volatiles of *O. kilimandscharicum* species from Nyeri County (OKI-NYR). Three major chemical constituents namely *p*-cymene [**41**] (peak 4) (5.80%), eucalyptol [**22**] (peak 6) (19.93%) and linalool [**27**] (peak 13) (19.2%) characterized the fresh volatiles of OKI-NYR. Minor chemical constituents such as estragole [**37**] (peak 17) (3.02%), *p*-ethylacetophenone [**68**]

(peak 19) (3.95%), *p*-methoxyacetophenone [**69**] (peak 22) (3.85%), (*E*)-β-caryophyllene [**35**] (peak 25) (3.37%), (*E*)-β-farnesene [**73**] (peak 27) (2.38%) and germacrene-D [**47**] (peak 29) (2.61%) among others, were also present in fresh volatiles of OKI-NYR (Figure 4.10).

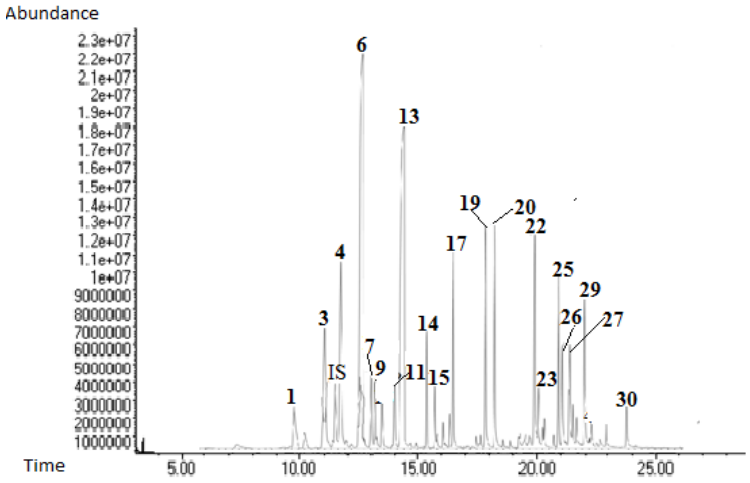
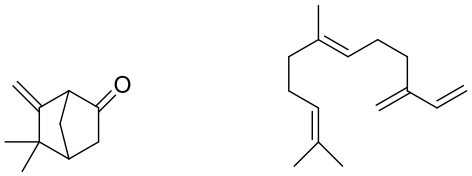


Figure 4.10: Total ion chromatogram of fresh volatiles of *O. kilimandscharicum* (Nyeri County)



(**72**) (**73**)

Fresh volatiles of *O. kilimandscharicum* species from Kirinyaga (OKI-KRN) and Nyeri (OKI-NYR) were characterized by monoterpenoids (49.1-56.1%), sesquiterpenoids (10.2-12.3%), benzenoids (16.7-25.7%) and non-terpenoids (1.1%). Eucalyptol [**22**] (5.2-19.9%) and *o*-cymene [**29**] (5.3-5.8%) occurred as major compounds of fresh volatile of both OKI-KRN and OKI-NYR. In addition to the two compounds, limonene [**23**] (6.1%) and camphor [**21**] (19.4%) also occurred as respective major compounds of OKI-KRN and OKI-NYR fresh volatiles. 6-Camphenone [**72**] (3.46%) was identified as a unique marker of OKI-KRN fresh volatiles.

4.3.3 Chemical composition of *O. lamiifolium* fresh volatiles

A total of twenty-nine chemical constituents were identified in fresh volatiles of *O. lamiifolium* species from both Nyandarua and Nakuru Counties (Table 4.7).

Table 4.7: Chemical constituents of *O. lamiifolium* fresh volatiles

GC Peak	RI	Identity of the compound	Amount in %	
			Nyandarua	Nakuru
1	932	α-pinene	1.88	1.29
2	974	β-pinene	-	1.06
3	976	β-Sabinene	-	15.73
4	980	1-Octen-3-ol	2.88	4.64
5	995	3-Octanal	-	4.41
6	1002	α-phellandrene	18.69	11.92
7	1008	δ-3-carene	1.16	0.89
8	1022	*o*-cymene	2.71	1.48
9	1024	Limonene	7.65	-
10	1044	(*E*)-β-ocimene	7.04	-
11	1055	1,3-Diethyl benzene	1.68	1.87
12	1074	Sabinene hydrate	-	4.69
13	1097	Nonan-2-ol	-	2.82
14	1106	1,2-Diethyl benzene	-	2.00
15	1110	Octen-3-yl acetate	3.05	3.75
16	1163	4-ethylbenzaldehyde	1.23	1.61
17	1174	2-ethylbenzaldehyde	-	0.55
18	1273	*p*-ethylacetophenone	4.73	6.61
19	1290	*p*-methoxyacetophenone	4.76	6.59
20	1370	Carvacrol acetate	2.80	-
21	1409	α-Gurjunene	1.77	-
22	1417	(*E*)-β-Caryophyllene	0.72	0.88
23	1484	Germacrene-D	-	1.47
24	1492	(*Z*)-β-Guaiene	1.20	-
25	1494	Bicyclogermacrene	2.70	-
26	1524	Δ-Cadinene	0.58	-
27	1574	Germacrene-D-4-ol	0.55	0.66
28	1435	1,2-Diacetylbenzene	1.11	2.23
29	1451	1,4-Diacetylbenzene	1.33	1.85
		Monoterpenoids	**36.42**	**15.16**
		Sesquiterpenoids	**7.52**	**2.13**
		Benzenoids	**17.54**	**24.79**
		Non-terpenoids	**8.73**	**15.52**

Twenty-one chemical compounds were identified in fresh volatiles of *O. lamiifolium* from Nyandarua County (OLA-NYD). Fresh volatiles of OLA-NYD were characterized by α-phellandrene [**31**] (peak 6) (18.69%), limonene [**23**] (peak 9) (7.65%) and (*E*)-β-ocimene [**20**] (peak 10) (7.04%). Octen-3-yl acetate [**74**] (peak 15) (3.05%), *p*-ethylacetophenone [**68**] (peak 18) (4.73%), *p*-methoxyacetophenone (**69**) (peak 19) (4.76%), 1-octen-3-ol [**75**] (peak 4) (2.88%) and bicyclogermacrene [**76**] (peak 25) (2.70%) among others, were present as minor compounds of fresh volatiles of OLA-NYD (Figure 4.11).

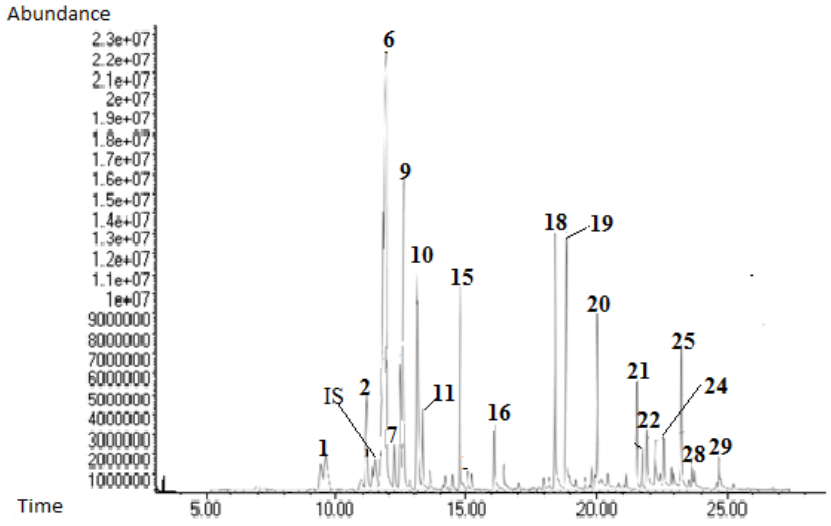


Figure 4.11: Total ion chromatogram of fresh volatiles of *O. lamiifolium* (Nyandarua County)

Twenty-two chemical constituents were identified in fresh volatiles of *O. lamiifolium* species from Nakuru County (OLA-NKU). Five major constituents namely β-sabinene [**30**] (peak 3) (15.73%), α-phellandrene [**31**] (peak 6) (11.92%), *p*-ethylacetophenone [**68**] (peak 18) (6.61%), *p*-methoxyacetophenone [**69**] (peak 19) (6.59%) and sabinene hydrate [**77**] (peak 12) (4.69%) characterized the fresh volatiles of OLA-NKU. Minor constituents such

as 1, 2 diacetylbenzene [**70**] (peak 28) (2.23%) and octen-3-yl acetate [**74**] (peak 15) (3.75%) among others, were also identified in OLA-NKU (Figure 4.12).

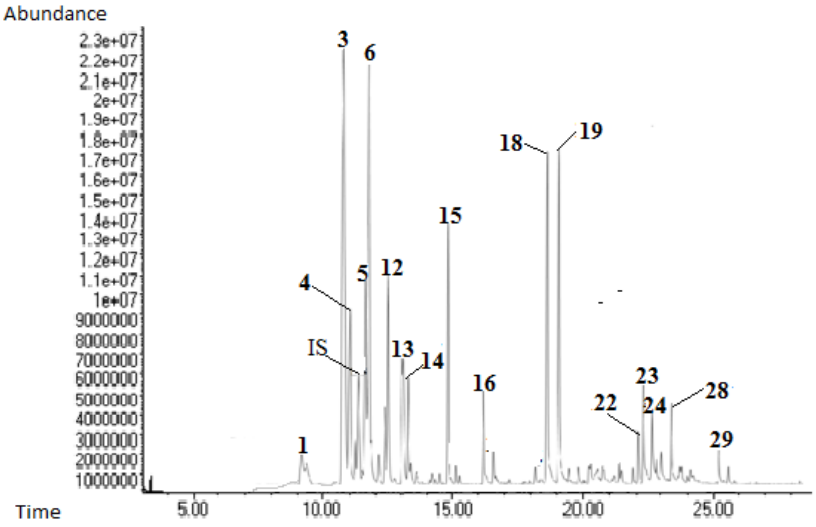
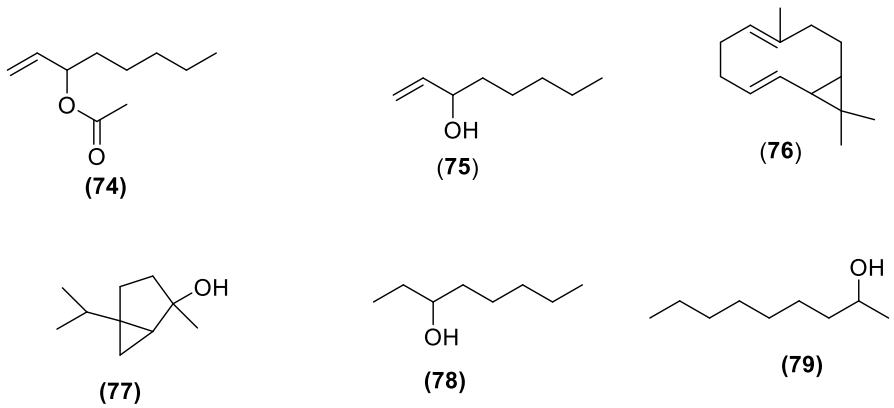


Figure 4.12: Total ion chromatogram of fresh volatiles of *O. lamiifolium* (Nakuru County)



Monoterpenoid (15.2-36.4%), benzenoid (17.5-24.8%), sesquiterpenoid (2.1-7.5%) and non-terpenoid (8.8-15.5%) were identified in fresh volatiles of *O. lamiifolium* species from both Nyandarua (OLA-NYD) and Nakuru (OLA-NKU). α-Phellandrene [**31**] (11.9-18.7%) occurred as a major compound of fresh volatiles of both OLA-NYD and OLA-NKU. On the other hand, (*E*)-β-ocimene [**20**] (7.0%) was reported as a major compound of OLA-

NYD fresh volatiles though absent in OLA-NKU fresh volatiles. 3-Octanol [**78**] (4.4%) and nonan-2-ol [**79**] (2.8%) were unique markers of OLA-NKU fresh volatiles.

4.4 Chemical composition of smoldered volatiles of selected *Ocimum* species

GC-MS analyses of smoldered volatiles of selected *Ocimum* species led to identification of a total of forty-three, fifty and twenty-two chemical constituents in *O. kilimandscharicum, O. lamiifolium* and *O. kenyense* smoldered volatiles, respectively. α-Phellandrene [**31**] (0.81-12.76%) was identified in smoldered volatiles of all investigated *Ocimum* species in varying concentrations. Chemical composition of smoldered volatiles of *O. kilimandscharicum, O. lamiifolium* and *O. kenyense* species are presented in Table 4.8, 4.9 and 4.10. Control and blank chromatograms for smoldered volatiles is presented in Appendix 8 (B and C).

4.4.1 Chemical composition of *O. kenyense* smoldered volatiles

A total of twenty-two chemical constituents were identified in smoldered volatiles of *O. kenyense* species from both Laikipia and Nyeri Counties (Table 4.8).

Table 4.8: Chemical constituents of *O. kenyense* smoldered volatiles

GC Peak	RI	Identity of the compound	Amount in %	
			Laikipia	**Nyeri**
1	932	α-Pinene	-	0.93
2	974	β-Pinene	0.46	3.27
3	988	β-Myrcene	0.48	1.26
4	1002	α-Phellandrene	1.23	1.04
5	1026	Eucalyptol	10.71	27.93
6	1141	Camphor	-	2.19
7	1144	(2)-Bornanone	1.23	-
8	1074	Sabinene hydrate	1.46	1.79
9	1315	*p*-Vinyl-guaicol	0.88	-
10	1417	(*E*)-β-Caryophyllene	4.32	1.54
11	1452	α-Humuulene	4.88	4.44
12	1505	β-Bisabolene	7.47	6.70
13	1608	Humulene epoxide II	0.86	-
14	1028	*o*-Cymene	6.61	-
15	1195	Estragole	18.32	30.93
16	1242	Anethole	7.65	-
17	1247	Chavicol	3.31	6.27
18	1346	2,6-dimethoxy phenol	1.19	-
19	1403	Methyl eugenol	1.12	-
20	2132	Linoleic acid	1.25	-
21	2257	8-isopropyl-1,3-dimethylphenathrene	0.98	-
22	2808	Squalene	0.48	-
		Monoterpenoids	**14.11**	**39.62**
		Sesquiterpenoids	**17.36**	**14.47**
		Benzenoids	**38.20**	**34.20**
		Triterpenoids	**0.48**	**-**
		Non-terpenoids	**2.47**	**0.83**

Twenty compounds were identified in smoldered volatiles of *O. kenyense* species from Laikipia County (OKE-LKP). Eucalyptol [**22**] (peak 5) (10.71%), *o*-cymene [**29**] (peak 16) (6.61%), estragole [**37**] (peak 15) (18.32%), anethole [**43**] (peak 16) (7.65%) and β-bisabolene [**53**] (peak 14) (7.47%) were identified as major compounds of OKE-LKP smoldered volatiles. Several minor components such as (*E*)-β-caryophyllene [**35**] (peak 12)

(4.32%), α-humulene [**51**] (peak 50) (4.88%) and chavicol [**50**] (peak 17) (3.31%), among others, were also identified in OKE-LKP smoldered volatiles (Figure 4.13).

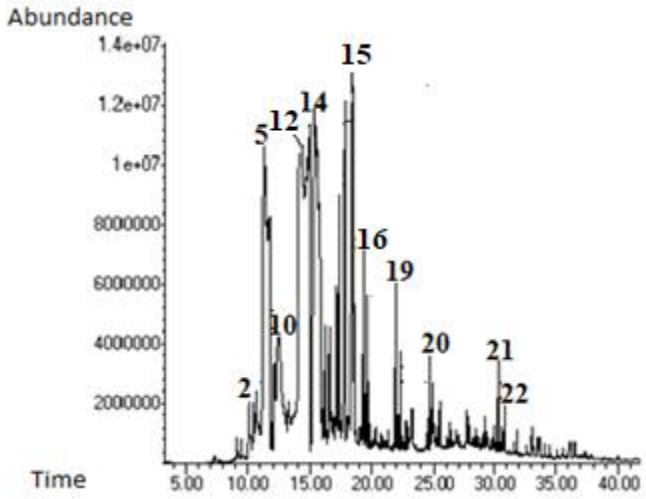


Figure 4.13: Total ion chromatogram of smoldered volatiles of *O. kenyense* (Laikipia County)

Twelve chemical compounds were reported in smoldered volatiles of aerial parts of *O. kenyense* species from Nyeri County (OKE-NYR). Five major compounds namely eucalyptol [**22**] (peak 5) (27.93%), estragole [**37**] (peak 15) (30.93%), chavicol [**50**] (peak 17) (6.27%), α-humulene [**51**] (peak 13) (4.44%) and β-bisabolene [**52**] (peak 14) (6.70%) characterized OKE-LKP smoldered volatiles. Sabinene hydrate [**77**] (peak 10) (1.79%), camphor [**21**] (peak 6) (2.19%) and (*E*)-β-caryophyllene [**35**] (peak 10) (1.54%) among others, were present as minor chemical compounds of smoldered volatiles of OKE-NYR (Figure 4.14).

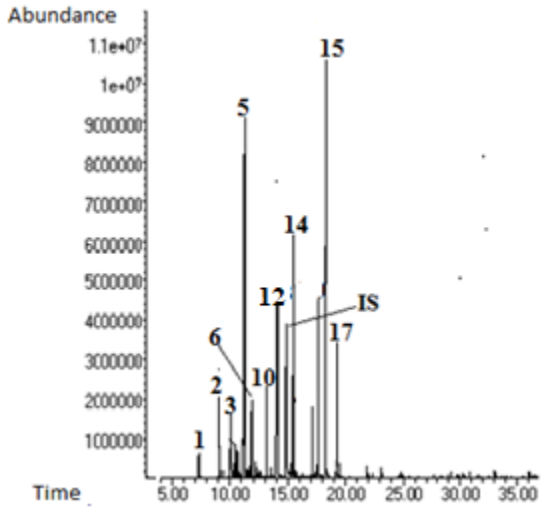
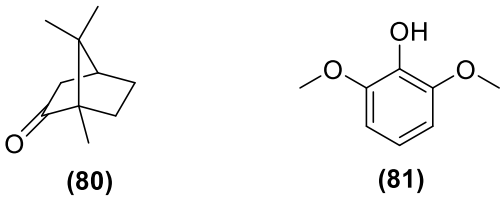


Figure 4.14: Total ion chromatogram of smoldered volatiles of *O. kenyense* (Nyeri County)

The chemical composition of the various constituents of smoldered volatiles of *O. kenyense* species from Laikipia (OKE-LKP) and Nyeri (OKE-NYR) included monoterpenoids (14.1-39.6%), sesquiterpenoids (14.5-17.5%), benzenoids (34.2-38.2%), triterpenoids (0.5%) and non-terpenoids (0.8-2.5%). Estragole [**37**] (18.3-30.9%), eucalyptol [**22**] (10.7-27.9%) and β-bisabolene [**52**] (6.7-7.5%) occurred as major compounds of smoldered volatiles of *O. kenyense* species from both Laikipia (OKE-LKP) and Nyeri (OKE-LKP). (2)-Bornanone [**80**] (peak 7) (1.23%) and 2,6-dimethoxyphenol [**81**] (peak 18) (1.19%) were identified as unique markers of OKE-LKP smoldered volatiles.



(80) **(81)**

4.4.2 Chemical composition of *O. kilimandscharicum* smoldered volatiles

A total of forty-three chemical constituents were identified in smoldered volatiles of *O. kilimandscharicum* species from both Kirinyaga and Nyeri Counties (Table 4.9)

Table 4.9: Chemical constituents of *O. kilimandscharicum* smoldered volatiles

GC Peak	RI	Identity of the compound	Amount in %	
			Kirinyaga	Nyeri
1	932	α-Pinene	1.56	-
2	946	Camphene	4.80	-
3	974	β-Pinene	1.68	0.92
4	988	β-Myrcene	1.98	0.77
5	1002	α-Phellandrene	2.31	0.81
6	1008	3-carene	-	2.36
7	1018	α-Terpinene	0.71	-
8	1024	Limonene	13.36	3.62
9	1026	Eucalyptol	-	10.66
10	1027	Sylvestrene	-	1.38
11	1044	(*E*)-β-Ocimene	1.14	6.05
12	1050	Phenol	0.67	-
13	1083	Fenchone	2.36	4.45
14	1122	2-Carene	-	2.51
15	1141	Camphor	17.32	12.62
16	1162	Borneol	-	0.84
17	1174	Terpinen-4-ol	-	1.40
18	1195	Estragole	9.81	3.45
19	1224	Nerol	2.15	-
20	1247	Chavicol	-	0.72
21	1249	Geraniol	10.67	6.74
22	1315	*p*-Vinyl-guaicol	-	0.61
23	1350	Eugenol	0.51	-
24	1351	α-Cubebene	-	0.74
25	1359	Neryl acetate	2.39	-
26	1374	β-Bourbonene	-	0.85
27	1389	β-Cubebene	-	1.52
28	1391	α-Copaene	1.18	1.80
29	1409	α-Gurjunene	-	0.71
30	1416	β-Copaene	-	4.41
31	1417	(*E*)-β-Caryophyllene	5.38	8.00
32	1452	α-Humulene	-	2.07
33	1454	(*E*)-β-Farnesene	4.38	3.94
34	1484	Germacrene-D	1.78	-
35	1485	α-Amorphene	-	1.20

Table 4.9 Continued.

GC Peak	RI	Identity of the compound	Amount in %	
			Kirinyaga	**Nyeri**
36	1491	α-Bisabolene	0.76	-
37	1505	β-Bisabolene	1.82	3.32
38	1963	Hexadecanoic acid	-	0.83
39	1993	1,3-dimethylphenathrene	-	0.51
40	2132	Linoleic acid	-	0.87
41	1886	Farnesyl acetate	-	0.59
42	2808	Squalene	-	0.56
43	2987	α-Tocopherol	-	0.57
		Monoterpenoids	**64.40**	**54.00**
		Sesquiterpenoids	**14.12**	**23.55**
		Benzenoids	**10.99**	**4.17**
		Triterpenoids	**-**	**0.56**
		Non-terpenoids	**2.39**	**2.76**

Twenty-two compounds were identified in smoldered volatiles of *O. kilimandscharicum* species from Kirinyaga County (OKI-KRN). Camphene [**24**] (peak 2) (4.80%), limonene [**23**] (peak 8) (13.36%), camphor [**21**] (peak 15) (17.32%), estragole [**37**] (peak 18) (9.81%), geraniol [**17**] (peak 21) (10.67%) and (*E*)-β-caryophyllene [**35**] (peak 30) (5.38%) characterized OKI-KRN smoldered volatiles. Nerol [**19**] (peak 19) (2.15%), α-phellandrene [**31**] (peak 5) (2.31%) and (*E*)-β-farnesene [**73**] (peak 32) (4.38%) among others, were also present as minor compounds of OKI-KRN smoldered volatiles (Figure 4.15).

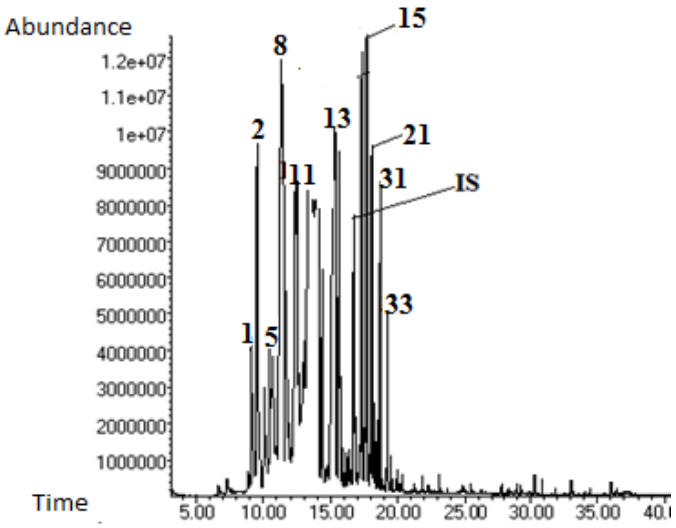


Figure 4.15: Total ion chromatogram of smoldered volatiles of O. *kilimandscharicum* (Kirinyaga County)

Thirty-four chemical constituents were reported in smoldered volatiles of *O. kilimandscharicum* species from Nyeri County (OKI-NYR). Geraniol [**17**] (peak 21) (6.74%), (*E*)-β-ocimene [**20**] (peak 14) (6.05%), camphor [**21**] (peak 24) (12.62%), eucalyptol [**22**] (peak 9) (10.66%) and (*E*)-β-caryophyllene [**35**] (peak 30) (8.00%) were identified as major components of smoldered volatiles of OKI-NYR. Minor chemical constituents such as limonene [**23**] (peak 8) (3.62%), estragole [**37**] (peak 18) (3.45%), fenchone [**82**] (peak 29) (4.41%), (*E*)-β-farnesene [**73**] (peak 32) (3.94%) and β-bisabolene [**52**] (peak 36) (3.32%) among others were reported as minor constituents of OKI-NYR smoldered volatiles (Figure 4.16).

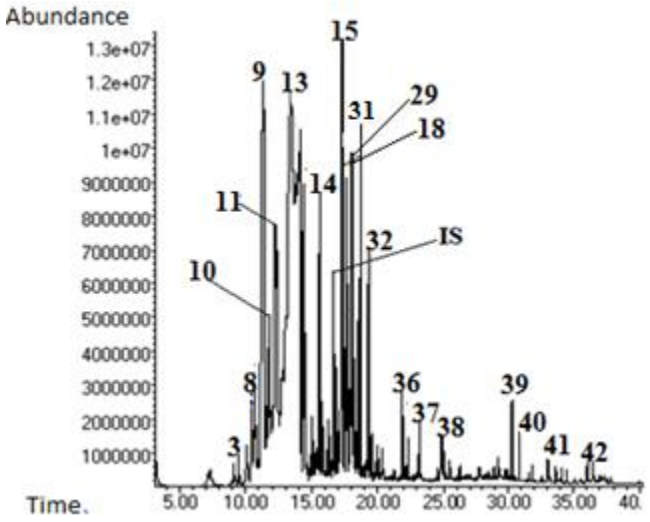
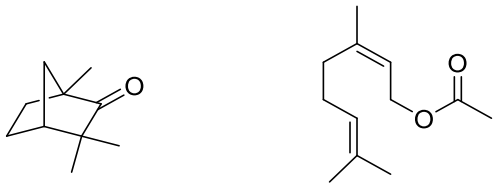


Figure 4.16: Total ion chromatogram of smoldered volatiles of *O. kilimandscharicum* (Nyeri County)

Smoldered volatiles of *O. kilimandscharicum* from both Kirinyaga (OKI-KRN) and Nyeri (OKI-NYR) Counties were dominated by monoterpenoids (61.2-61.3%), sesquiterpenoids (14.1-23.5%), benzenoids (4.2-10.9%), triterpenoids (0.6%) and non-terpenoids (2.4-2.8%). Camphor [**21**] (12.6-17.3%), geraniol [**17**] (6.7-10.6%) and (*E*)-β-caryophyllene [**35**] (5.4-8.0%) were major compounds of OKI-KRN and OKI-NYR smoldered volatiles. Neryl acetate [**83**] (2.4%) was identified as a unique marker of OKI-KRN smoldered volatiles.



(82) **(83)**

4.4.3 Chemical composition of *O. lamiifolium* smoldered volatiles

A total of fifty chemical constituents were identified in smoldered volatiles of *O. lamiifolium* species from both Nyandarua and Nakuru Counties (Table 4.10).

Table 4.10: Chemical constituents of *O. lamiifolium* smoldered volatiles

GC Peak	RI	Identity of the compound	Amount in %	
			Nyandarua	Nakuru
1	932	α-Pinene	-	0.52
2	974	β-Pinene	-	0.51
3	1002	α-Phellandrene	8.41	12.76
4	1028	*o*-Cymene	-	7.96
5	1032	β-Phellandrene	-	1.09
6	1044	(*E*)-β-Ocimene	5.14	-
7	1068	4-ethyl-o-xylene	5.41	-
8	1076	*p*-Cymenene	1.13	1.40
9	1113	3,4-dimethylbenzyl alcohol	-	2.94
10	1135	(E)-Sabinol	-	1.30
11	1141	Camphor	3.03	0.76
12	1157	Propenyl-2-phenol	0.78	-
13	1195	Estragole	1.13	-
14	1249	Geraniol	0.98	-
15	1315	*p*-Vinyl-guaicol	3.59	0.53
16	1351	α-Cubebene	-	0.67
17	1374	β-Bourbonene	2.85	4.63
18	1391	α-Copaene	-	1.12
19	1409	α-Gurjunene	4.89	2.03
20	1416	β-Copaene	1.64	2.11
21	1417	(*E*)-β-Caryophyllene	3.90	-
22	1451	Spirolepechinene	-	2.09
23	1461	Allo-aromanderene	2.09	-
24	1465	Dauca-5,8-diene	-	1.42
25	1472	γ-Muurolene	-	1.92
26	1484	Germacrene-D	2.94	6.35
27	1494	Bicyclogermacrene	4.01	2.69
28	1508	6-Epi-shybunol	2.78	0.81
29	1524	Δ-Cadinene	4.48	-
30	1548	Palustrol	0.61	-
31	1576	Spathulenol	2.66	-
32	1586	Germacra-1,6-diene-5-ol	1.27	4.04
33	1590	Viridiflorol	-	2.14
34	1602	Ledol	0.75	0.72
35	1652	α-Cadinol	-	0.67
36	1675	Cadalene	0.51	-
37	1685	Oplopanone	-	0.64
38	1688	Shybunol	1.36	-
39	1886	Farnesyl acetone	-	0.57
40	1967	Sclarene	-	0.75
41	1993	1,3-dimethylphenathrene	1.00	0.96
42	2080	Heptadecanoic acid	0.71	-

Table 4.10 Continued.

GC Peak	RI	Identity of the compound	Amount in %	
			Nyandarua	Nakuru
43	2158	Octadecanoic acid	0.72	-
44	2176	Retene	0.75	0.88
45	2198	Benzo (a) phenazine	0.57	-
46	2257	8-isopropyl-1,3-dimethylphenathrene	-	0.96
47	2274	Dehydroabietan	-	1.06
48	2808	Squalene	0.60	0.59
49	2987	α-Tocopherol	0.59	0.81
50	3376	α-Amyrin	0.47	0.67
		Monoterpenoids	**19.20**	**18.87**
		Sesquiterpenoids	**37.69**	**33.42**
		Benzenoids	**3.04**	**12.30**
		Triterpenoids	**1.66**	**2.07**
		Non-terpenoids	**17.85**	**13.17**

Thirty-two chemical compounds were identified in smoldered volatiles of *O. lamiifolium* from Nyandarua County (OLA-NYD). Three major compounds namely; α-phellandrene [**31**] (peak 3) (8.41%), (*E*)-β-ocimene [**20**] (peak 6) (5.14%) and 4-ethyl-o-xylene [**84**] (peak 7) (5.41%) characterized OLA-NYD smoldered volatiles. Minor compounds such *p*-vinyl guaicol [**85**] (peak 15) (3.59%), β-bourbonene [**86**] (peak 17) (2.83%) and α-gurjunene [**63**] (peak 19) (4.89%) were also identified in smoldered volatiles of OLA-NYD.

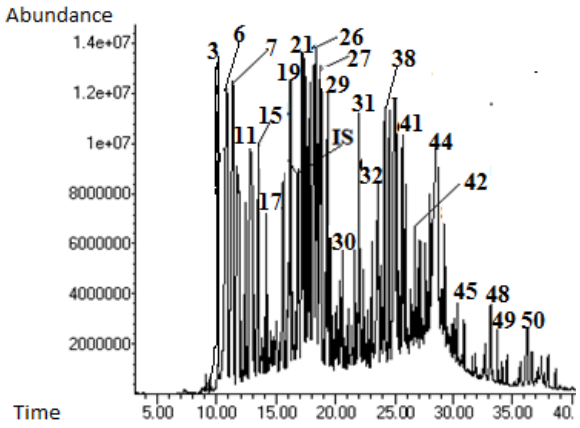


Figure 4.17: Total ion chromatogram of smoldered volatiles of *O. lamiifolium* (Nyandarua County)

Thirty-five chemical compounds were reported in smoldered volatiles of *O. lamiifolium* species from Nakuru County (OLA-NKU). Three major compounds namely α-phellandrene [**31**] (peak 3) (12.76%), *o*-cymene [**29**] (peak 4) (7.96%) and germacrene-D [**47**] (peak 26) (6.05%) characterized OLA-NKU smoldered volatiles (Figure 4.18).

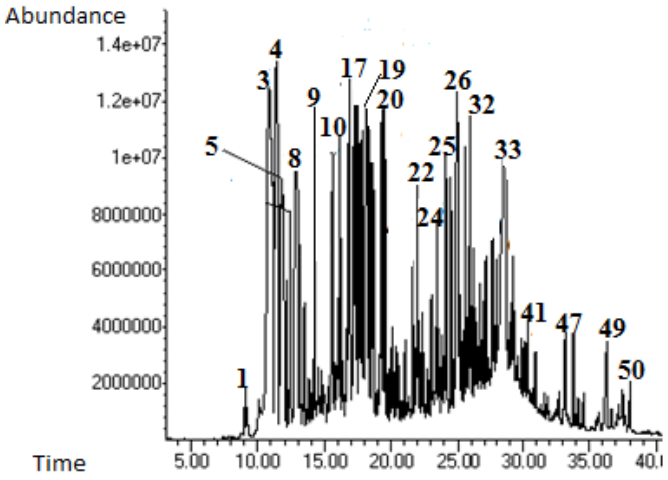
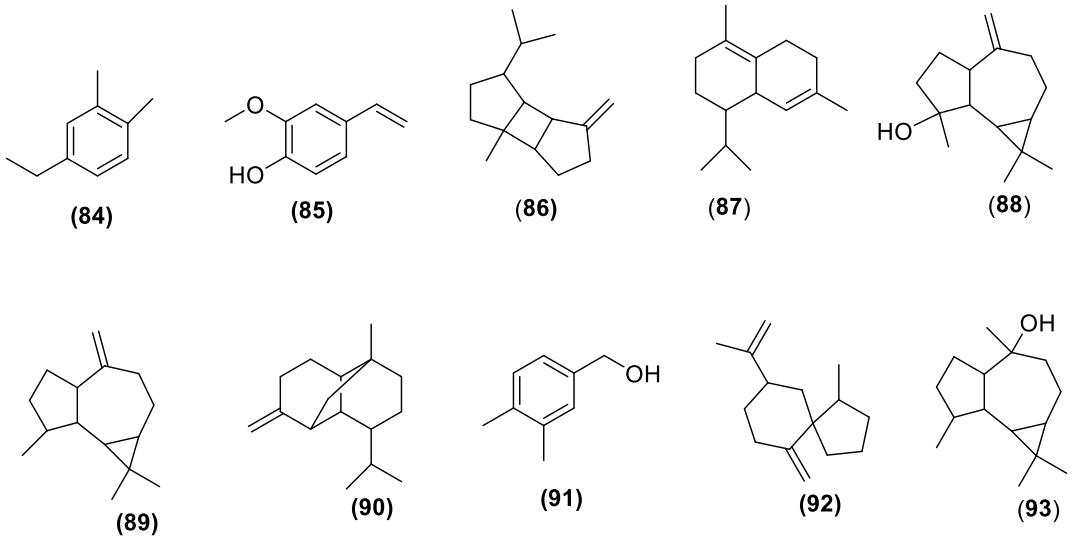


Figure 4.18: Total ion chromatogram of smoldered volatiles of *O. lamiifolium* (Nakuru County)

The chemical composition of smoldered volatiles of *O. lamiifolium* species from Nyandarua (OLA-NYD) and Nakuru (OLA-NKU) comprised of monoterpenoids (18.9-19.2%), sesquiterpenoids (33.4-37.7%), benzenoids (3.0-12.3%), triterpenoids (1.7-2.1%) and non-terpenoids (13.2-17.6%). α-Phellandrene [**31**] (8.4-12.8%) was identified as a major chemical constituent of OLA-NYD and OLA-NKU. Unique markers of OLA-NYD smoldered volatiles were identified as Δ-cadinene [**87**] (4.48%), spathulenol [**88]** (2.66%) and allo-aromandrene [**89**] (2.09%) (Figure 4.17). Similarly, *O. lamiifolium*-Nakuru (OLA-NKU) smoldered volatiles were marked by β-Copaene [**90**] (peak 20) (2.11%), 3,4-dimethylbenzyl alcohol [**91**] (2.94%), spirolepichinene [**92**] (2.09%) and viridiflorol [**93**] (2.14%).

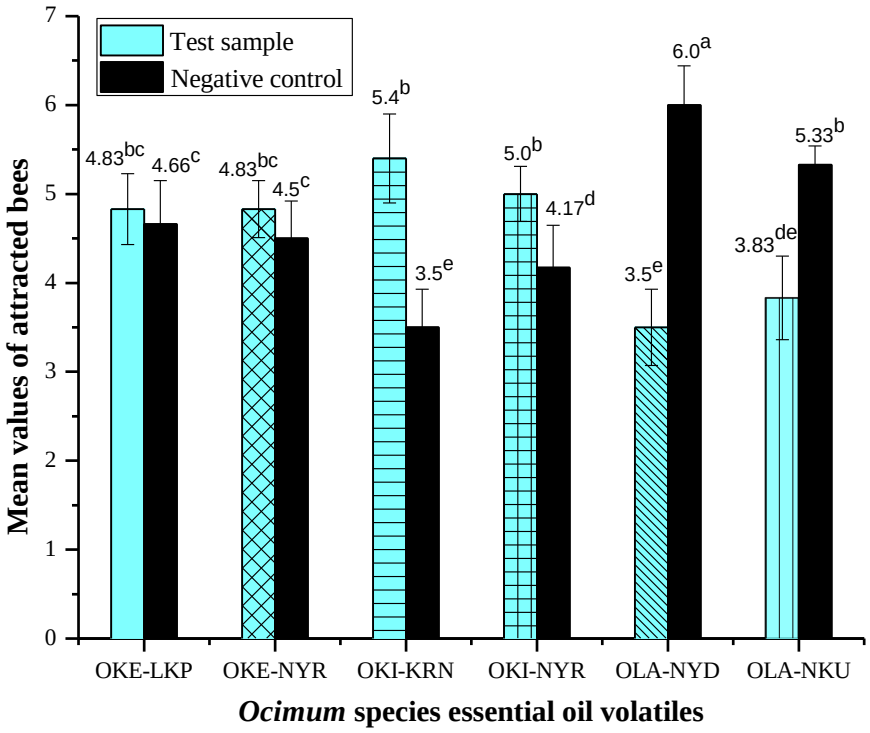


4.5 Responses of honey bees to essential oil volatiles of selected *Ocimum* species

The mean values of honey bees attracted to essential oils of selected *Ocimum* species alongside negative control (an empty vial with a capillary tube) in Y-tube olfactometer are given in Figure 4.19. From the results, there were significant variations ($p<0.05$), in attraction of honey bees to essential oils of *O. kenyense, O. kilimandscharicum* and *O. lamiifolium*. Essential oils of all investigated *Ocimum* species with exception of *O. lamiifolium* were significantly ($p<0.05$) more attractive than the negative control. *Ocimum kilimandscharicum* species from Kirinyaga (OKI-KRN) and Nyeri (OKI-NYR) essential oils were most attractive to honey bees with respective mean values of attracted bees of 5.40 ± 0.50 and 5.00 ± 0.31.

Essential oils of *O. kenyense* species from both Laikipia (OKE-LKP) and Nyeri (OKE-NYR) had equivalent mean values of attracted bees of at 4.83 ± 0.40 and 4.83 ± 0.31, respectively. Essential oils of *O. lamiifolium* species from both Nakuru (OLA-NKU) and

Nyandarua (OLA-NYD) exhibited low activity against honey bees with mean values of attracted bees of 3.83 ± 0.47 and 3.50 ± 0.43, respectively in comparison with respective controls with mean values of 6.00 ± 0.44 and 5.33 ± 0.21. There were significant differences in honey bee attractant activity of essential oils of the same species from two different agro ecological zones with exception of *O. lamiifolium* species (Figure 4.19).



*Mean values followed by different letters are significantly different at p<0.05; N=6

Figure 4.19: Honey bee attractant activity of *Ocimum* species' essential oil volatiles

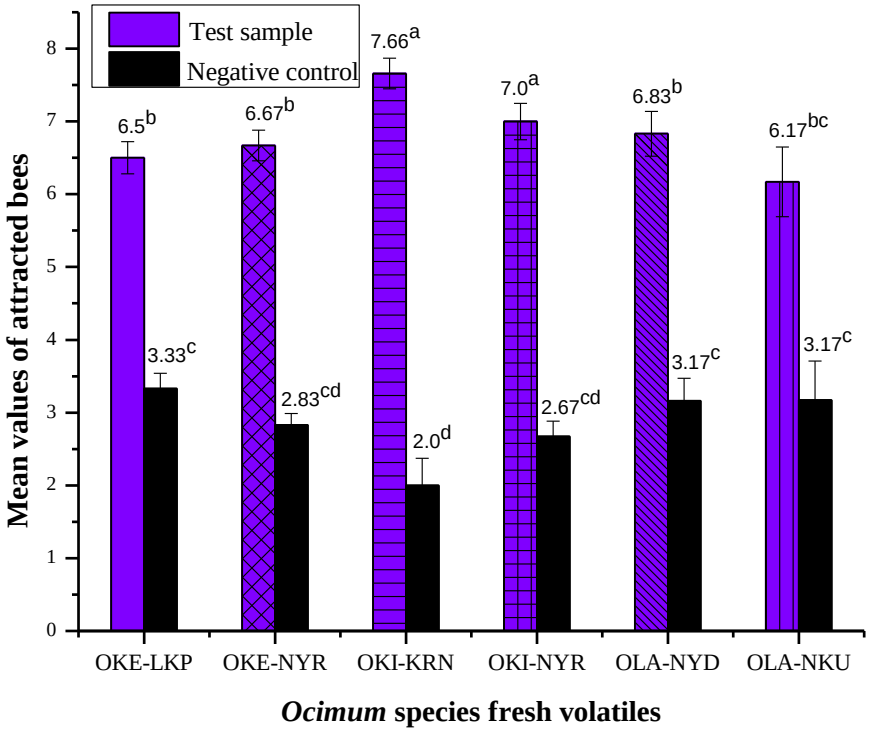
4.6 Responses of honey bees to fresh volatiles of selected *Ocimum* species

Fresh volatiles of *O. kenyense, O. kilimandscharicum* and *O. lamiifolium* species were assayed for bee attractant activity in Y-tube olfactometer against choices of negative and positive (bee wax) controls. In addition, olfactometric bioassays of two species component

blends of fresh volatiles as well as blends of fresh volatiles and bee wax were also conducted.

4.6.1 Responses of honey bees to fresh volatiles and negative control

Mean values of honey bees attracted to selected *Ocimum* species fresh volatiles alongside negative control (a white net tied in a similar shape as the plant material to eliminate visual effects) are presented in Figure 4.20. From the results, honey bee preferences for fresh volatiles of *O. kenyense, O. kilimandscharicum* and *O. lamiifolium* species were significantly ($p<0.05$) higher than their preferences for negative control. Honey bee attractant activity of fresh volatiles of the three species varied significantly ($p<0.05$). Fresh volatiles of *O. kilimandscharicum* species from Kirinyaga (OKI-KRN) and Nyeri (OKI-NYR) were most attractive to honey bees with respective mean values of attracted bees of 7.66 ± 0.21 and 7.00 ± 0.25.



*Mean values followed by different letters are significantly different at p<0.05; N=6

Figure 4.20: Honey bee attractant activity of *Ocimum* species' fresh volatiles

Means values of attracted bees to *O. kenyense* species' fresh volatiles from Laikipia (OKE-LKP) and Nyeri (OKE-NYR) were 6.50 ± 0.22 and 6.67 ± 0.21, respectively. On the other hand, O. *lamiifolium* fresh volatiles from Nyandarua (OLA-NYD) and Nakuru (OLA-NKU) had mean values of attracted bees of 6.83 ±0.31 and 6.17 ± 0.48, respectively. However, there were no significant differences in bee attractant activity of fresh volatiles of the same *Ocimum* species from two agro ecologial zones with exception of *O. lamiifolium* species.

4.6.2 Responses of honey bees to fresh volatiles and positive control

Fresh volatiles of three *Ocimum* species found to be highly attractive to honey bees in assays against negative control were tested against positive control (bee wax). The most attractive species included *O. kenyense*-Nyeri (OKE-NYR), *O. kilimandcharicum*-Kirinyaga (OKI-KRN) and *O. kilimandscharicum*-Nyeri (OKI-NYR). The *Ocimum* species' fresh volatiles to be tested were selected on basis of their mean values of attracted bees ranging between 6.67 ± 0.21 and 7.66 ± 0.21, respectively (Figure 4.20). The mean values of attracted bees were either equivalent or greater than bee wax's value of 6.67 ± 0.33. Results of mean values of bees attracted to *Ocimum* species fresh volatiles tested alongside a positive control (bee wax) are presented in Table 4.11.

Table 4.11: Mean values of honey bees attracted to *Ocimum* species fresh volatiles and positive control

	(Mean ± SD) bees attracted to treatments	
***Ocimum* species**	**Test sample**	**Positive control**
OKE-NYR	4.83 ± 0.17^{b}	4.16 ± 0.31^{c}
OKI-KRN	5.33 ± 0.21^{a}	3.67 ± 0.26^{d}
OKI-NYR	5.33 ± 0.33^{a}	4.33 ± 0.42^{bc}

NB: Means in a column followed by different letters are significantly different at $p<0.05$, with untreated (positive control) as reference treatment

Honey bees' preferences for all investigated fresh volatiles were significantly ($p<0.05$) higher as compared to positive control. Bee attractant activity of OKI-KRN and OKI-NYR was equivalent with respective mean values of attracted bees of 5.33 ± 0.21 and 5.33 ± 0.33. However, bee attractant activity of OKI-KRN and OKI-NYR was significantly different from that of OKE-NYR whose mean value of attracted bees was reported as 4.83 ± 0.17, ($p<0.05$).

4.6.3 Responses of honey bees to fresh volatiles-bee wax blends

Ocimum fresh species volatiles of *O. kenyense*-Nyeri (OKE-NYR), *O. kilimandcharicum*-Kirinyaga (OKI-KRN) and *O. kilimandscharicum*-Nyeri (OKI-NYR) were blended with bee wax. The blended samples of *Ocimum* species volatiles and bee wax namely OKE-NYR-BWF, OKI-KRN-BWF and OKI-NYR-BWF, were tested against negative control, which comprised of a clean stone with a similar shape as that of the test sample wrapped in white net to minimize visual effects. Mean values of honey bees attracted to blends of fresh volatiles of selected *Ocimum* species and bee wax against negative and positive contols are presented in Table 4.12 and 4.13, respectively.

Table 4.12: Mean values of honey bees attracted to fresh volatiles-bee wax blends of volatiles and negative control

Blends of *Ocimum* species fresh volatiles + bee wax	(Mean ± SD) bees attracted to treatments	
	Test sample	Negative control
OKE-NYR-BWF	6.16 ± 0.17^{b}	3.50 ± 0.31^{c}
OKI-KRN-BWF	6.67 ± 0.21^{a}	2.83 ± 0.26^{d}
OKI-NYR-BWF	6.67 ± 0.21^{a}	3.33 ± 0.21^{c}
BEE WAX	6.67 ± 0.33^{a}	3.16 ± 0.17^{cd}

NB: Means in a column followed by different letters are significantly different at $p<0.05$, with untreated (negative) control as reference treatment

Key: OKE-NYR-BWF=*O. kenyense* (NYR) + Bee wax; OKI-KRN-BWF=*O. kilimandscharicum* (KRN) + Bee wax; OKI-NYR-BWF=*O. kilimandscharicum* (NYR) + Bee wax; F=Fresh volatiles

From the results, honey bees' preferences for blends of *Ocimum* species' fresh volatiles and bee wax and bee wax alone were significantly ($p<0.05$) higher than their preferences for the negative control. Bee attractant activity of blends bee wax and fresh volatiles of *O. kilimandscharicum*-Kirinyaga species (OKI-KRN-BWF) and *O. kilimandscharicum*-Nyeri species (OKI-NYR-BWF) was equivalent with a mean value of attracted bees of 6.67 ± 0.21.

Attraction honey bees to OKI-KRN-BWF and OKI-NYR-BWF blends did not vary significantly from their attraction to bee wax alone with a mean value of attracted bees of 6.67 ± 0.33. However, a blend of bee wax and fresh volatiles of *O. kenyense*-Nyeri (OKE-NYR-BWF) blend was significantly less attractive to bees with a mean value of attracted bees of 6.16 ± 0.17 ($p<0.05$), as compared to OKI-KRN-BWF, OKI-NYR-BWF and bee wax alone (Table 4.12).

Table 4.13: Mean values of honey bees attracted to fresh volatiles-bee wax blends of volatiles and positive control

Blends of *Ocimum* species fresh volatiles and bee wax	(Mean ± SD) bees attracted to treatments	
	Test sample	Positive control
OKE-NYR-BWF	4.83 ± 0.17^{c}	4.67 ± 0.21^{c}
OKI-KRN-BWF	5.33 ± 0.21^{bc}	4.50 ± 0.22^{c}
OKI-NYR-BWF	5.83 ± 0.16^{b}	3.67 ± 0.21^{d}

NB: Means in a column followed by different letters are significantly different at $p<0.05$, with positive control as reference treatment

Key: OKE-NYR-BWF=*O. kenyense* (NYR) + Bee wax; OKI-KRN-BWF=*O. kilimandscharicum* (KRN) + Bee wax; OKI-NYR-BWF=*O. kilimandscharicum* (NYR) + Bee wax; F=Fresh volatiles

Individual blends of bee wax and fresh volatiles of *O. kilimandscharicum*-Nyeri (OKI-NYR-BWF) and *O. kilimandscharicum*-Kirinyaga (OKI-KRN-BWF) were significantly more attractive to honey bees as compared to bee wax (positive control). Respective mean values of bees attracted to OKI-KRN-BWF and OKI-NYR-BWF blends were 5.33 ± 0.21 and 5.83 ± 0.16. However, there was no significant variation in attraction of honey bees to blend of bee wax and fresh volatiles of *O. kenyense*-Nyeri (OKE-NYR-BWF) and positive control. Mean values of bees attracted to OKE-NYR-BWF and bee wax (positive control) were 4.83 ± 0.17 and 4.67 ± 0.21, respectively (Table 4.13).

4.6.4 Responses of honey bees to two component blends of *Ocimum* species' fresh volatiles

Each of the three blends comprising of two *Ocimum* species' fresh volatiles was tested against negative control. Results of mean values of attracted bees are presented in Table 4.14.

Table 4.14: Mean values of honey bees attracted to two component blends of *Ocimum* species' fresh volatiles and negative control

Blends of *Ocimum* species fresh volatiles	(Mean ± SD) bees attracted to treatments	
	Test sample	Negative control
KIK-NKF	6.67 ± 0.21^{a}	2.83 ± 0.26^{d}
KEK-NKF	5.67 ± 0.21^{b}	4.00 ± 0.26^{cd}
KEK-NNF	5.00 ± 0.21^{bc}	4.33 ± 0.17^{c}

NB: Means in a column followed by different letters are significantly different at $p<0.05$, with untreated (negative) control as reference treatment

Key: KIK-NKF=*O. kilimandscharicum* (KRN) + *O. kilimandscharicum* (NYR); KEK-NKF=*O. kilimandscharicum* (KRN) + *O. kenyense* (NYR) and KEK-NNF=*O. kilimandscharicum* (NYR) + *O. kenyense* (NYR)

Honey bees' preferences for the three *Ocimum* species fresh volatiles' blends were significantly higher ($p<0.05$) as compared to negative control. Fresh volatile blend comprising of *O. kilimandscharium* species from Kirinyaga and Nyeri (KIK-NKF) was signifantly ($p<0.05$) more attractive with a mean value of attracted bees of 6.67 ± 0.21 as compared to blends comprising of *O. kenyense*-Nyeri and *O. kilimandscharicum*-Kirinyaga and Nyeri (KEK-NKF and KEK-NNF) (Table 4.14).

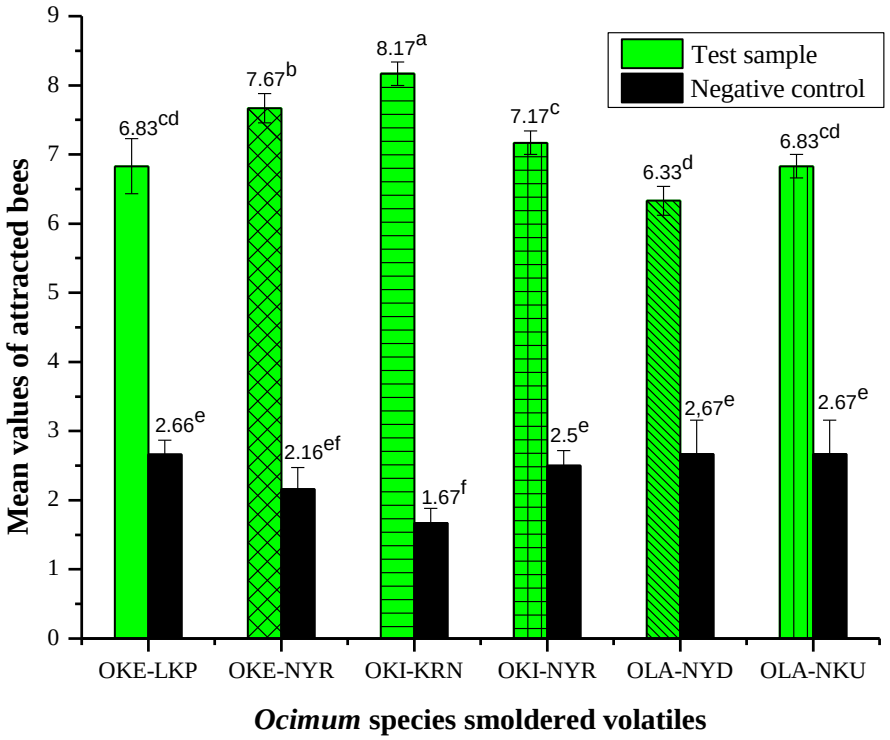
4.7 Responses of honey bees to smoldered volatiles of selected *Ocimum* species

Mean values of bees attracted to choices of individual smoldered volatiles, two species component blends and smoldered volatiles and bee wax blends against negative and positive controls are presented in this section.

4.7.1 Responses of honey bees to smoldered volatiles and negative control

The mean values of honey bees attracted to smoldered volatiles of selected *Ocimum* species and negative control (clean stainless-steel bowl with smoldering charcoal) are presented in Figure 4.21. Honey bees' preferences for smoldered volatiles were significantly different ($p<0.05$) as compared to their preference for negative control. Smoldered volatiles of *O. kilimandscharicum*-Kirinyaga (OKI-KRN) and Nyeri (OKI-NYR) mean values of attracted bees were 8.17 ± 0.17 and 7.17 ± 0.17, respectively.

The mean values of bees attracted to *O. kenyense* species' smoldered volatiles from Laikipia (OKE-LKP) and Nyeri (OKE-NYR) were 6.83 ± 0.40 and 7.67 ± 0.21, respectively. On the other hand, mean values of bees attracted to smoldered volatiles of *O. lamiifolium* species from Nyandarua (OLA-NYD) and Nakuru (OLA-NKU) 6.33 ± 0.21 and 6.83 ± 0.48, respectively. Honey bee preferences for all selected *Ocimum* species' smoldered volatiles varied significantly ($p<0.05$), with agro ecological zone of origin.



*Mean values followed by different letters are significantly different at p<0.05; N=6

Figure 4.21: Honey bee attractant activity of *Ocimum* species' smoldered volatiles

4.7.2 Responses of honey bees to smoldered volatiles and positive control

Smoldered volatiles of three *Ocimum* species found to be highly attractive to honey bees when tested against negative control, were also tested against bee wax (positive control). The three species populations namely *O. kenyense*-Nyeri (OKE-NYR), *O. kilimandcharicum*-Kirinyaga (OKI-KRN) and *O. kilimandscharicum*-Nyeri (OKI-NYR) were selected on basis of their mean values of attracted bees ranging between 7.17 ± 0.17 and 8.17 ± 0.17, respectively (Figure 4.21). The mean values of attracted bees were either equivalent or greater than bee wax's mean of 6.67 ± 0.33. Results of mean values of bees

attracted to *Ocimum* species smoldered volatiles tested against positive control (bee wax) are presented in Table 4.15.

Table 4.15: Mean values of honey bees attracted to smoldered volatiles and positive control

	(Mean ± SD) bees attracted to treatments	
***Ocimum* species**	**Test sample**	**Positive control**
OKE-NYR	6.67 ± 0.21^{b}	3.16 ± 0.31^{e}
OKI-KRN	7.33 ± 0.24^{a}	2.67 ± 0.21^{f}
OKI-NYR	6.17 ± 0.31^{c}	3.50 ± 0.22^{d}

NB: Means in a column followed by different letters are significantly different at $p<0.05$, with untreated (positive) control as reference treatment

Honey bees' preferences for all investigated fresh volatiles were significantly ($p<0.05$), higher as compared to the positive control. Bee attractant activity of *O. kenyense*-Nyeri (OKE-NYR), *O. kilimandscharicum*-Kirinyaga (OKI-KRN) and *O. kilimandscharicum*-Nyeri (OKI-NYR) was significantly different with respective mean values of attracted bees of 6.67 ± 0.21, 7.33 ± 0.24 and 6.17 ± 0.31 ($p<0.05$) (Table 4.15).

4.7.3 Responses of honey bees to smoldered volatiles-bee wax blends

Ocimum species smoldered volatiles of *O. kenyense*-Nyeri (OKE-NYR), *O. kilimandscharicum*-Kirinyaga (OKI-KRN) and *O. kilimandscharicum*-Nyeri (OKI-NYR) were blended with bee wax. The blended sample of *Ocimum* species smoldered volatiles and bee wax was tested against negative control, which comprised of a clean stone with a similar shape as that of the test sample placed in a clean stainless-steel container. Mean values of honey bees attracted to blends of smoldered volatiles of selected *Ocimum* species and bee wax against negative and positive controls were as presented in Table 4.16 and 4.17, respectively.

Table 4.16: Mean values of honey bees attracted to smoldered volatiles-bee wax blends and negative control

Blends of *Ocimum* species smoldered volatiles and bee wax	(Mean ± SD) bees attracted to treatments	
	Test sample	Negative control
OKE-NYR-BWS	6.33 ± 0.21^{b}	3.00 ± 0.31^{d}
OKI-KRN-BWS	7.00 ± 0.26^{a}	2.83 ± 0.26^{e}
OKI-NYR-BWS	6.50 ± 0.22^{bc}	3.16 ± 0.31^{d}
BEE WAX	6.67 ± 0.33^{a}	3.16 ± 0.17^{cd}

NB: Means in a column followed by different letters are significantly different at $p<0.05$, with untreated (negative) control as reference treatment

Key: OKE-NYR-BWS=*O. kenyense* (NYR) + Bee wax; OKI-KRN-BWS=*O. kilimandscharicum* (KRN) + Bee wax; OKI-NYR-BWS=*O. kilimandscharicum* (NYR) + Bee wax; S=Smoldered volatiles

From the results, honey bees' preferences for blends of *Ocimum* species' smoldered volatiles and bee wax were significantly ($p<0.05$) higher than their preferences for the negative control. Bee attractant activity of blends of bee wax and smoldered volatiles of *O. kenyense*-Nyeri (OKE-NYR-BWS), *O. kilimandscharicum*-Kirinyaga (OKI-KRN-BWS) and *O. kilimandscharicum*-Nyeri (OKI-NYR-BWS) varied significantly with respective mean value of attracted bees of 6.33 ± 0.21, 7.00 ± 0.26 and 6.50 ± 0.22 ($p<0.05$). Attraction of honey bees to OKI-KRN-BWS and bee wax alone did not vary significantly with respective mean values of attracted bees of 7.00 ± 0.26 and 6.67 ± 0.33 (Table 4.16).

Table 4.17: Mean values of honey bees attracted to smoldered volatiles-bee wax blends and positive control

Blends of *Ocimum* species smoldered volatiles and bee wax	(Mean ± SD) bees attracted to treatments	
	Test sample	Positive control
OKE-NYR-BWS	6.67 ± 0.21^{a}	3.00 ± 0.25^{cd}
OKI-KRN-BWS	7.00 ± 0.25^{a}	2.83 ± 0.30^{d}
OKI-NYR-BWS	5.83 ± 0.16^{b}	3.67 ± 0.21^{c}

NB: Means in a column followed by different letters are significantly different at $p<0.05$, with untreated (positive) control as reference treatment

Key: OKE-NYR-BWS=*O. kenyense* (NYR) + Bee wax; OKI-KRN-BWS=*O. kilimandscharicum* (KRN) + Bee wax; OKI-NYR-BWS=*O. kilimandscharicum* (NYR) + Bee wax; S=Smoldered volatiles

Individual blends of bee wax and smoldered volatiles of *O. kenyense*-Nyeri (OKI-NYR-BWS), *O. kilimandscharicum*-Nyeri (OKI-NYR-BWS) and *O. kilimandscharicum*-Kirinyaga (OKI-KRN-BWS) were significantly more attractive to honey bees as compared to bee wax (positive control). Respective mean values of bees attracted to OKE-NYR-BWS, OKI-KRN-BWS and OKI-NYR-BWS blends were 6.67 ± 0.21, 7.00 ± 0.25 and 5.83 ± 0.16 (Table 4.17)

4.7.4 Responses of honey bees to two component blends of *Ocimum* species' smoldered volatiles

Three two component blends of *Ocimum* species' smoldered volatiles were tested against negative control and mean values of attracted bees presented in Table 4.18.

Table 4.18: Means of honey bees attracted to smoldered volatiles-bee wax blends and negative control

Smoldered volatiles' blend	(Mean ± SD) bees attracted to treatments	
	Test sample	Negative control
KIK-NKS	6.83 ± 0.17^{a}	3.00 ± 0.26^{f}
KEK-NKS	4.00 ± 0.26^{d}	6.00 ± 0.25^{b}
KEK-NNS	4.50 ± 0.15^{cd}	4.83 ± 0.11^{c}

NB: Means in a column followed by different letters are significantly different at $p<0.05$, with untreated (negative) control as reference treatment

Key: KIK-NKS=*O. kilimandscharicum* (KRN) + *O. kilimandscharicum* (NYR); KEK-NKS=*O. kilimandscharicum* (KRN) + *O. kenyense* (NYR) and KEK-NNS=*O. kilimandscharicum* (NYR) + *O. kenyense* (NYR)

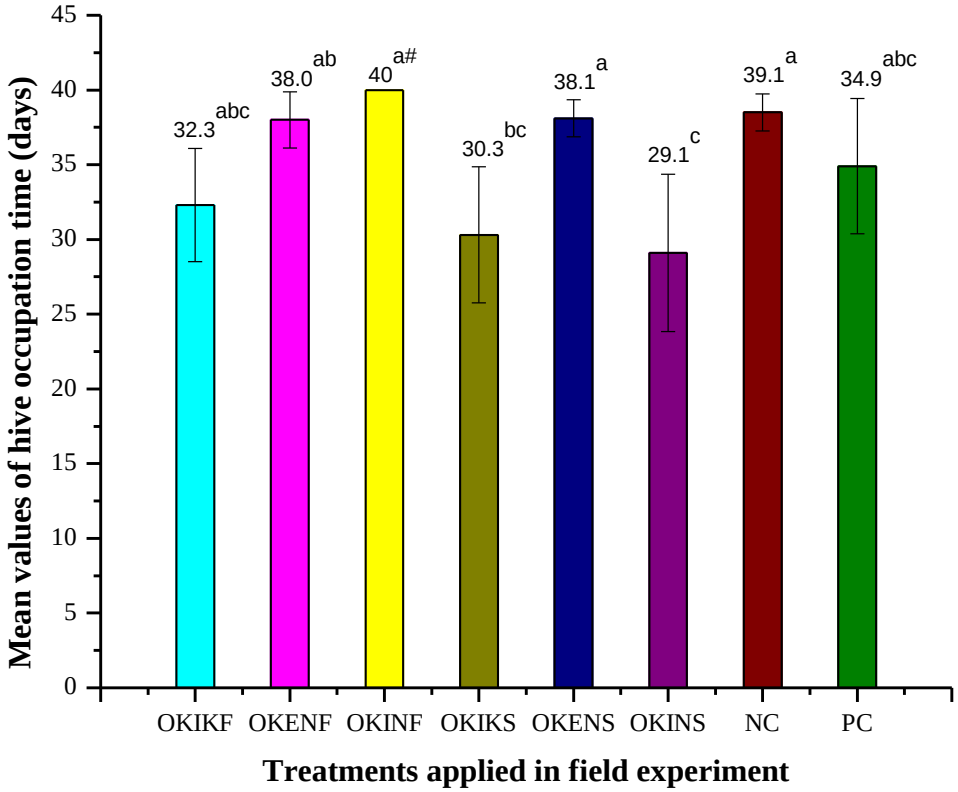
Smoldered volatile blend comprising of *O. kilimandscharium* species from Kirinyaga and Nyeri (KIK-NKF) was significantly ($p<0.05$) more attractive to bees with a mean value of attracted bees of 6.83 ± 0.21 as compared to blends comprising of *O. kenyense*-Nyeri and *O. kilimandscharicum* from Kirinyaga (KEK-NKS) and Nyeri (KEK-NNS). Honey bees'

preferences for blends comprising of *O. kenyense*-Nyeri, *O. kilimandscharicum*-Kirinyaga and *O. kilimandscharicum*-Nyeri (KEK-NKS and KEK-NNS) were significantly lower ($p<0.05$) as compared to negative control with respective mean values of attracted bees of 4.00 ± 0.26 and 4.50 ± 0.15.

4.8 Responses of honey bees to *Ocimum* species volatiles in field bioassay

Smoldered volatiles demonstrated by *O. kilimandscharicum* from Nyeri and Kirinyaga agro ecological zones (OKINS and OKIKS) exhibited a significantly higher ($p<0.05$) bee luring activity in field as compared to positive control. Mean hive occupation times of OKINS, OKIKS and positive control (PC) were 29.1, 30.3 and 34.9 days respectively. However, there was no significant difference in bee luring activity of fresh volatiles of *O. kilimandscharicum*-Kirinyaga (OKIKF) and bee wax with respective hive occupation times of 32.3 and 34.9 days.

Bee attractant activity of smoldered volatiles of *O. kenyense*-Nyeri (OKENS), fresh volatiles of *O. kilimandscharicum*-Nyeri (OKINF) and negative control (NC) did not vary significantly. Application of the three treatments resulted in respective hive occupation times of 38.1, 40.0 and 39.1 days being recorded. However, application of fresh volatiles of *O. kilimandscharicum*-Nyeri (OKINF) did not result in hive occupation in all months of the year and in all experimental sites (Figure 4.22).



*Mean values followed by different letters are significantly different at p<0.05; N=8; #-no hive occupation occured

Key: OKIK-*O. kilimandscharicum* (Kirinyaga), OKENF-*O. kenyense* (Nyeri) OKINF-*O. kilimandscharicum* (Nyeri), F-Fresh volatiles, S-Smoldered volatiles, NC-Negative control and PC-Positive control

Figure 4.22: Bee attractant activity of various *Ocimum* species' volatiles in field bioassay

Effects of hive placement site were observed in this study with site 6 (S6) and site (S8) being significantly more ($p<0.05$) preferred by honey bees as compared to all other sites with respective hive occupation times of 31.38 and 30.63 days. S7 was least preferred by honey bees with mean occupation time of 39.4 days while preference for sites S1, S2, S3, S4 and S5 did not vary significantly with mean hive occupation time ranging from 33.0 to 38.0 days (Figure 4.23).

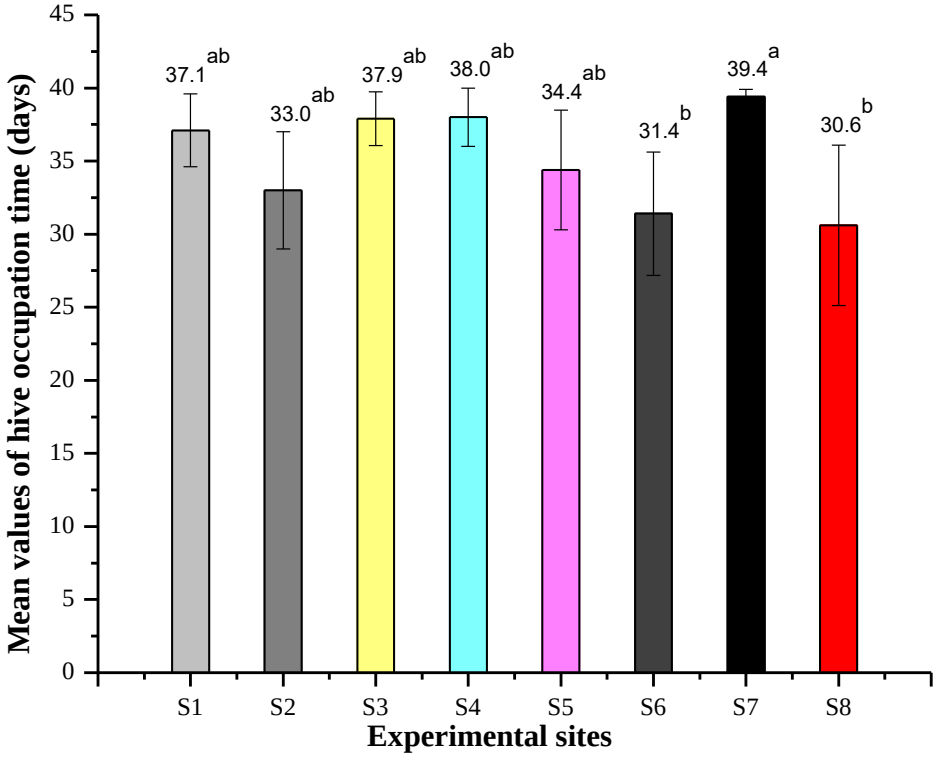
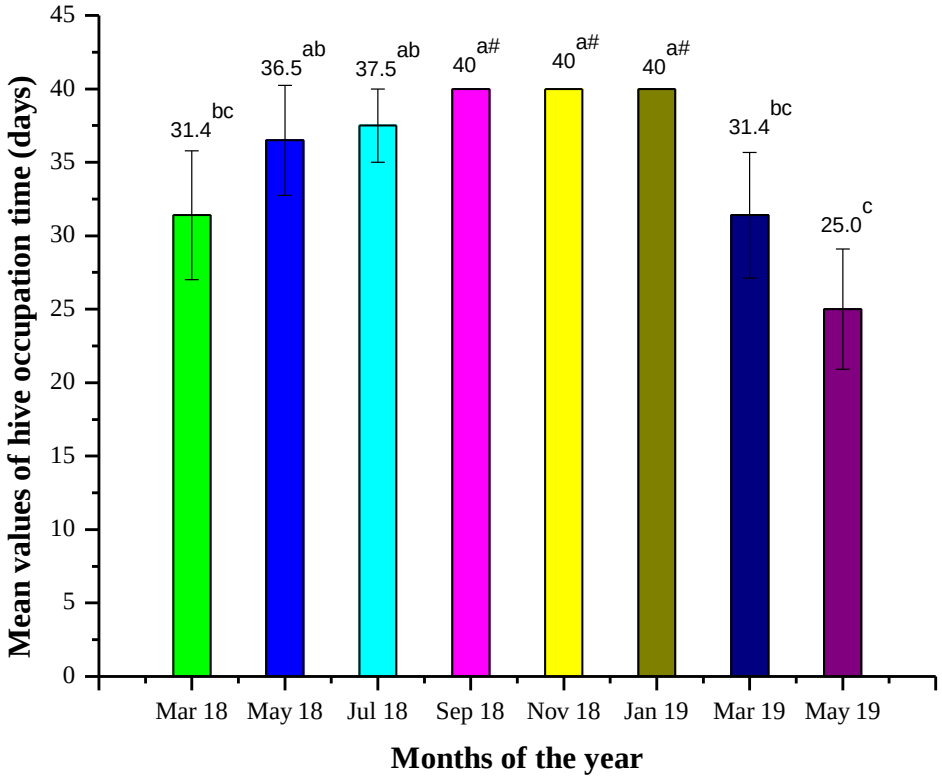


Figure 4.23: Effects of experimental site on bee attractant activity of *Ocimum* species volatiles

In the months of March, May and July 2018 as well as March and April 2019, respective hive occupation times of 31.4, 36.5, 37.5 31.4 and 25.0 days were recorded. The rates of hive occupation were significant ($p<0.05$) in the months of March 2018 and 2019 as well as in April 2019. A hive occupation rate of 75% (six out of eight hives) was recorded in the month of April 2019. In the months of March 2018 and 2019, hive occupation rates of 50% (four out of eight hives occupied hives) were recorded. However, mean hive occupation of 40.0 days was recorded in the months of September and November 2018 as well as in January 2019, an indication that no hive occupation during the stated period (Figure 4.24).



*Mean values followed by different letters are significantly different at p<0.05; N=8; #- no hive occupation occured

Figure 4.24: Effects of hive placement time on bee attractant activity of *Ocimum* species volatiles

4.9 Responses of honey bees to synthetic blends of *Ocimum* species' volatiles in olfactometric bioassay

Chemical composition of synthetic blends of fresh and smoldered volatiles of *O. kilimandscharicum* (Kirinyaga), *O. kilimandscharicum* (Nyeri) and *O. kenyense* (Nyeri) are presented in Table 4.19, 4.20 and 4.21, respectively.

Table 4.19: Chemical compositions of synthetic blends of *O. kilimandscharicum* (Kirinyaga) fresh and smoldered volatiles

Fresh volatiles synthetic blend			**Smoldered volatiles synthetic blend**		
Chemical component	**%**	**Wt/Vol (µg/ml)**	**Chemical component**	**%**	**Wt/Vol (µg/ml)**
Camphene	6.1	67.6	Camphene	4.8	58.1
β-pinene	2.0	19.2	Limonene	13.4	161.7
Eucalyptol	5.2	48.7	Camphor	17.3	209.6
Limonene	9.9	93.7	Nerol	2.2	26.0
o-cymene	5.3	50.0	Geraniol	10.7	122.0
(*E*)-β-ocimene	3.6	34.3	Estragole	9.8	118.8
Camphor	9.5	183.7	(*E*)-β-caryophyllene	5.4	63.2
Geraniol	3.7	34.7	(*E*)-β-farnesene	4.4	53.0
(*E*)-β-caryophyllene	4.1	38.8	Neryl acetate	2.4	28.9
(*E*)-β-farnesene	2.4	22.4			
p-ethylacetophenone	3.8	36.3			
p-methoxyacetophenone	3.9	36.6			
Total	**69.5**		**Total**	**70.4**	

Table 4.20: Chemical compositions of synthetic blends of *O. kilimandscharicum* (Nyeri) fresh and smoldered volatiles

Fresh volatiles synthetic blend			**Smoldered volatiles synthetic blend**		
Chemical component	**%**	**Wt/vol (µg/ml)**	**Chemical component**	**%**	**Wt/Vol (µg/ml)**
β-pinene	4.7	40.5	3-carene	2.4	25.6
o-cymene	5.8	49.7	Limonene	3.6	40.8
Eucalyptol	19.9	170.6	Eucalyptol	10.7	120.1
Linalool	19.2	164.4	(*E*)-β-ocimene	6.1	68.1
(*E*)-β-caryophyllene	3.4	28.9	Fenchone	4.5	234.7
(*E*)-β-farnesene	2.3	20.0	Camphor	12.6	142.1
Estragole	3.0	25.9	Geraniol	6.7	75.9
p-ethylacetophenone	3.9	33.8	(*E*)-β-caryophyllene	8.0	90.1
p-methoxyacetophenone	3.8	32.9	α-humulene	2.1	23.3
Eugenol	3.9	33.0	(*E*)-β-farnesene	3.9	44.4
Total	**70.02**		**Total**	**63.96**	

Table 4.21: Chemical compositions of synthetic blends of *O. kenyense* (Nyeri) fresh and smoldered volatiles

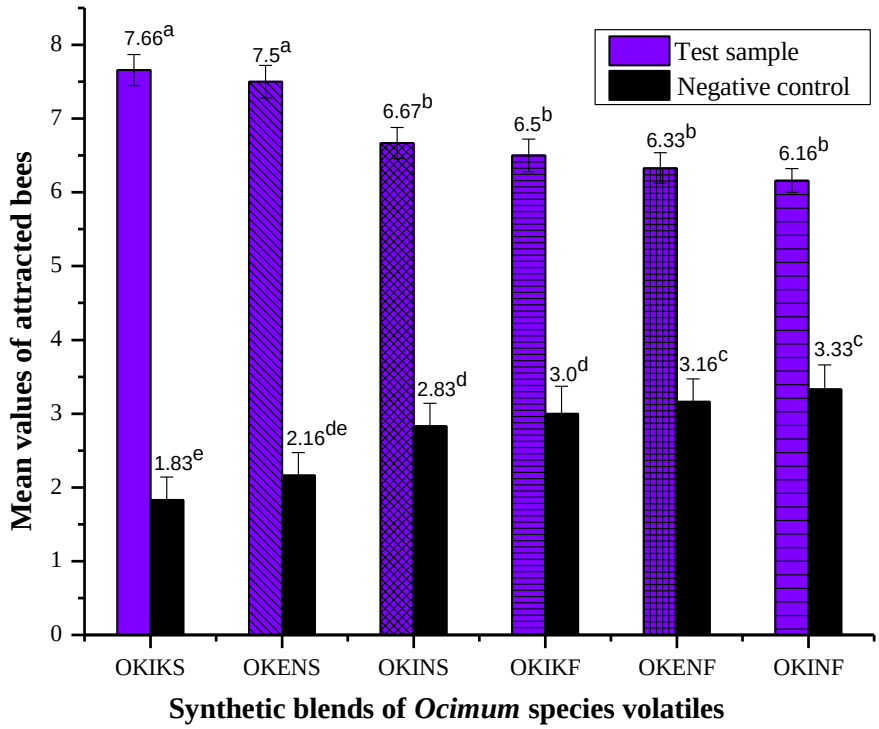
Fresh volatiles synthetic blend			**Smoldered volatiles synthetic blend**		
Chemical Component	**%**	**Wt/vol (µg/ml)**	**Chemical component**	**%**	**Wt/Vol (µg/ml)**
β-pinene	13.3	121.3	Eucalyptol	30.9	242.8
Eucalyptol	6.1	55.4	Camphor	2.2	17.1
p-cymene	5.1	46.3	α-humulene	4.4	34.9
α-humulene	4.9	44.3	Estragole	27.9	219.2
Ethyl isovalerate	7.6	69.2	β-pinene	3.3	25.7
p-ethylacetophenone	11.3	104.3	(*E*)-β-caryophyllene	1.5	12.1
p-methoxyacetophenone	11.7	106.2			
(*E*)-β-caryophyllene	1.3	11.9			
Total	**61.33**		**Total**	**70.30**	

4.9.1 Responses of honey bees to synthetic blends of *Ocimum* species' volatiles

Means of honey bees attracted to synthetic blends of *Ocimum* species' fresh and smoldered volatiles are presented in Figure 4.25. Honey bee preferences for synthetic blends of the most attractive *Ocimum* species volatiles, were significantly higher as compared to their preference for negative control. Smoldered volatiles of *O. kenyense*-Nyeri (OKENS) and *O. kilimandscharicum*-Kirinyaga (OKIKS) were significantly ($p<0.05$) more attractive to bees as compared to other four synthetic blends.

The mean values of bees attracted to OKENS and OKIKS were 7.50 ± 0.22 and 7.66 ± 0.21, respectively ($p<0.05$). However, bee attractant activity of synthetic blends of fresh volatiles of *O. kenyense*-Nyeri (OKENYF), *O. kilimandscharicum*-Kirinyaga (OKIKF) and *O. kilimandscharicum*-Nyeri (OKINF) as well as smoldered volatiles of *O. kilimandscharicum*-Nyeri (OKINS) did not vary significantly.

The mean values of bees attracted to the four synthetic blends ranged from 6.16 ± 0.16 to 6.67 ± 0.21. Three most attractive synthetic blends of *Ocimum* species namely *O. kenyense* (OKENYS), *O. kilimandscharicum* (OKINYS) and *O. kilimandscharicum* (OKIKRS) were tested against a positive control (bee wax in acetone) in a glass vial with a capillary tube.



*Mean values followed by different letters are significantly different at p<0.05; N=6

Figure 4.25: Honey bee activity of *Ocimum* species volatiles' synthetic blends

Means of honey bee attracted to synthetic blends of *Ocimum* species' smoldered volatiles are presented in Figure 4.26. Synthetic blends of smoldered volatiles of most attractive *Ocimum* species were significantly more preferable ($p<0.05$) to honey bees as compared to positive control. However, honey bees' preferences for OKIKRS; OKENYS and OKINYS

synthetic blends did not vary significantly, with respective mean values of attracted bees 6.83 ± 0.16, 6.67 ± 0.21 and 6.50 ± 0.22.

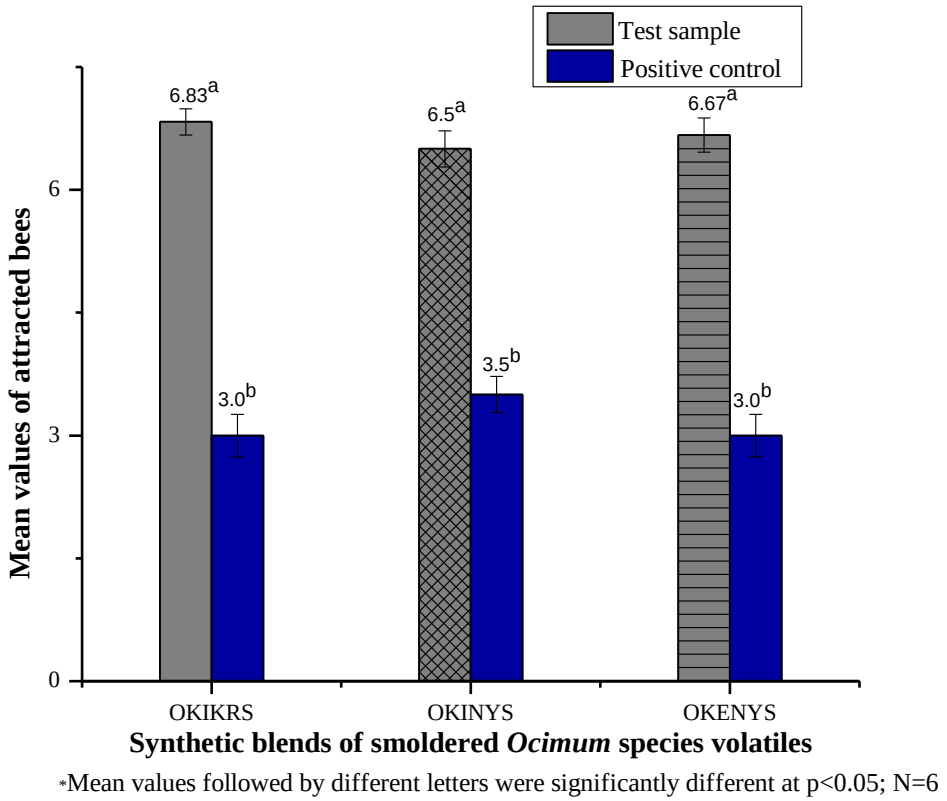


Figure 4.26: Honey bee attractant activity of synthetic blends of *Ocimum* species' smoldered volatiles against positive control

4.9.2 Responses of honey bees to synthetic blends of *Ocimum* species smoldered volatiles in subtraction assays

Mean values of bees attracted to specific subtraction blends of *O. kenyense* (NYR), *O. kilimandscharicum* (NYR) and *O. kilimandscharicum* (KRN) are presented in this section.

4.9.2.1 Responses of honey bees to specific synthetic blends of *O. kenyense* (NYR) smoldered volatiles

Means of bees attracted to synthetic blends of smoldered volatiles of *O. kenyense* (NYR) in subtraction bioassays are shown in Table 4.22.

Table 4.22: Means of honey bees attracted to synthetic blends of *O. kenyense*-Nyeri smoldered volatiles

Synthetic blend	Synthetic blend composition	(Mean ± SD) bees attracted to treatments	
		Test sample	Negative control
FB	Full blend*	6.67 ± 0.21^{b}	3.00 ± 0.26^{d}
A	Full blend – (camphor + β-pinene)	7.67 ± 0.21^{a}	2.00 ± 0.26^{e}
B	Camphor + β-pinene	4.67 ± 0.21^{c}	4.83 ± 0.17^{c}
C	A - Estragole	6.33 ± 0.21^{b}	3.17 ± 0.16^{d}
D	A - Eucalyptol	6.17 ± 0.17^{b}	3.50 ± 0.34^{d}
E	A- (*E*)-β-caryophyllene	7.50 ± 0.22^{a}	2.33 ± 0.33^{e}
F	A- α-humulene	7.66 ± 0.21^{a}	2.17 ± 0.31^{e}

NB: Means in a column followed by different letters are significantly different at $p<0.05$, with untreated (negative) control as reference treatment

Key: Full blend*=estragole + eucalyptol + camphor + β-pinene + α-humulene + (E)-β-caryophyllene

Subtraction of bicyclic monoterpene constituents (β-pinene and camphor) from the full synthetic blend of *O. kenyense*-Nyeri (OKE-NYR) smoldered volatiles led to a significant ($p<0.05$) increase in mean value of attracted bees from 6.67 ± 0.21 to 7.67 ± 0.21. When blend B comprising of camphor [**21**] and β-pinene [**55**] was assayed separately, it exhibited insignificant bee attractant activity with a mean value of attracted bees of 4.67 ± 0.21 against a negative control's mean value of 4.83 ± 0.17. However, subtraction of sesquiterpenoid constituents such as α-humulene [**51**] and (*E*)-β-caryophyllene [**35**] (blend E) did not result in a significant reduction in bee attractant activity of blend A (full blend – [camphor + β-pinene]) (Table 4.22).

In this study, blend A formed as a result exclusion of camphor [**21**] and β-pinene [**55**] from the full blend was used as a reference in assessing activity of individual chemical constituents of OKE-NYR smoldered volatiles' synthetic blend. Subtraction of eucalyptol and estragole from synthetic blend A of *O. kenyense*-Nyeri (OKE-NYR) smoldered volatiles led to a significant reduction in mean values of bees attracted from 7.67 ± 0.21 to 6.17 ± 0.17 and 6.33 ± 0.17 ($p<0.05$), respectively (Table 4.22). Therefore, eucalyptol and estragole were identified as key contributors to bee attractant activity of OKE-NYR smoldered volatiles' synthetic blends

4.9.2.2 Responses of honey bees to specific synthetic blends of *O. kilimandscharicum* (KRN) smoldered volatiles

Mean values of bees attracted to synthetic blends of smoldered volatiles of *O. kilimandscharicum* (KRN) in subtraction bioassays are shown in Table 4.23.

Table 4.23: Mean values of bees attracted to synthetic blends of *O. kilimandscharicum*-Kirinyaga species' smoldered volatiles

Synthetic blend	Synthetic blend composition	(Mean ± SD) bees attracted to treatments	
		Test sample	Negative control
FB	Full blend*	7.67 ± 0.22^{a}	1.83 ± 0.31^{i}
G	Full blend - (camphene + camphor)	7.83 ± 0.17^{a}	1.50 ± 0.22^{i}
H	Camphor + Camphene	4.50 ± 0.22^{e}	5.33 ± 0.21^{d}
I	G - Estragole	7.50 ± 0.22^{a}	2.17 ± 0.31^{gh}
J	G - (*E*)-β-caryophyllene	7.17 ± 0.17^{ab}	2.67 ± 0.21^{gh}
K	G - (*E*)-β-farnesene	6.67 ± 0.21^{bc}	2.83 ± 0.31^{fgh}
L	G – Limonene	6.67 ± 0.21^{bc}	3.00 ± 0.26^{fg}
M	G – Nerol	6.33 ± 0.21^{c}	3.50 ± 0.22^{f}
N	G - Neryl acetate	6.50 ± 0.22^{bc}	2.83 ± 0.31^{fgh}
O	G – Geraniol	6.16 ± 0.17^{c}	3.33 ± 0.33^{fg}

NB: Means in a column followed by different letters are significantly different at $p<0.05$, with untreated (negative) control as reference treatment

Key: Full blend*= camphene + limonene + camphor + nerol + geraniol + estragole + (*E*)-β-caryophyllene + (*E*)-β-farnesene + neryl acetate

From the results, subtraction of camphene [**24**] and camphor [**21**] from the full blend (FB) of *O. kilimandscharicum*-Kirinyaga (OKI-KRN) smoldered volatiles led to an insignificant increase in mean values of attracted bees from 7.67 ± 0.22 to 7.83 ± 0.17. Assay of a synthetic blend of camphene [**24**] and camphor [**21**] (blend G) exhibited insignificant bee attractant activity with a mean value of attracted bees of 4.50 ± 0.22 against a negative control's mean value of 5.33 ± 0.21. In subsequent bioassays, blend G excluding camphene [**24**] and camphor [**21**] was used as a reference in assessing individual activities of other chemical constituents of OKI-KRN smoldered volatiles' synthetic blend.

Subtraction of geraniol [**17**], nerol [**19**], neryl acetate [**83**], limonene [**23**], (E)-β-farnesene [**73**] and (*E*)-β-caryophyllene [**35**], individually from blend G of OKI-KRN led to a significant ($p<0.05$) reduction in bee attractant activity for each of the components, respectively (Table 4.23). The mean values of bees attracted to blend G decreased from 7.83 ± 0.17 to 6.33 ± 0.21 and 6.16 ± 0.17, with respective subtraction of nerol [**19**] and geraniol [**17**] (Table 4.23). The decline in mean values of attracted bees is an indication that bee attractant activity of OKI-KRN could be largely attributed to the presence of geraniol [**17**] and nerol [**19**] alongside other four chemical components highlighted above.

4.9.2.3 Responses of honey bees to specific synthetic blends of *O. kilimandscharicum* (NYR) smoldered volatiles

Means of bees attracted to synthetic blends of smoldered volatiles of *O. kilimandscharicum*-Nyeri (OKI-NYR) in subtraction bioassays are shown in Table 4.24.

Table 4.24: Means of honey bees attracted to synthetic blends of *O. kilimandscharicum*-Nyeri smoldered volatiles

Synthetic blend	Synthetic blend composition	(Mean ± SD) bees attracted to treatments	
		Test sample	**Negative control**
FB	Full blend*	6.67 ± 0.21^{a}	2.83 ± 0.31^{k}
P	Full blend - (Camphor + Fenchone)	6.83 ± 0.17^{ab}	2.83 ± 0.17^{k}
Q	Camphor + Fenchone	4.67 ± 0.33^{fg}	4.83 ± 0.31^{f}
R	P- 3-carene	6.50 ± 0.22^{ab}	3.33 ± 0.31^{ijk}
S	P- Estragole	6.33 ± 0.21^{abc}	3.50 ± 0.22^{ij}
T	P- α-humulene	6.17 ± 0.17^{bcd}	3.50 ± 0.22^{ij}
U	P- (*E*)-β-ocimene	5.83 ± 0.17^{cde}	3.67 ± 0.21^{hij}
V	P- Eucalyptol	5.67 ± 0.21^{de}	3.83 ± 0.16^{hi}
W	P- Geraniol	5.50 ± 0.22^{e}	4.16 ± 0.31^{gh}
X	P- (*E*)-β-farnesene	6.17 ± 0.17^{bcd}	3.50 ± 0.22^{ij}
Y	P- Limonene	6.17 ± 0.17^{bcd}	3.17 ± 0.31^{jk}
Z	P- (*E*)-β-caryophyllene	6.33 ± 0.21^{abc}	3.50 ± 0.22^{ij}

NB: Means in a column followed by different letters are significantly different at $p<0.05$, with untreated (negative) control as reference treatment

Key: Full blend*= 3-carene + limonene + eucalyptol + (*E*)-β-ocimene + fenchone + camphor + geraniol + (*E*)-β-caryophyllene + (*E*)-β-farnesene + α-humulene

Subtraction of bicyclic monoterpene compounds such as camphor and fenchone (blend P) from the full blend (FB) of *O. kilimandscharicum*-Nyeri (OKI-NYR) smoldered volatiles resulted in insignificant increase in mean value of attracted bees from 6.67 ± 0.21 to 6.83 ± 0.17. When blend Q comprising of camphor [**21**] and fenchone [**82**] was assayed, it exhibited insignificant bee attractant activity with a mean value of attracted bees of 4.67 ± 0.33 against negative control's mean value of 4.83 ± 0.31. Camphor [**21**] and fenchone [**82**] were excluded from subsequent bioassays.

Individual subtraction of geraniol [**17**], (*E*)-β-ocimene [**20**] and eucalyptol [**22**] from blend P of *O. kilimandscharicum*-Nyeri (OKI-NYR) led to a significant reduction in mean values

of attracted bees from 6.83 ± 0.17 to 5.50 ± 0.22, 5.67 ± 0.21 and 5.83 ± 0.17 ($p<0.05$) for each of the components, respectively. This observation is an indication that the three chemical constituents were key contributors to bee attractant activity of OKI-NYR synthetic blend alongside other constituents such as limonene [**23**], (*E*)-β-caryophyllene [**35**], estragole [**37**], α-humulene [**51**] and (*E*)-β-farnesene [**73**] (Table 4.24).

4.9.3 Responses of honey bees to synthetic blends of most active constituents

Three additive synthetic blends (SBAC1, SBAC2 and SBAC3) of the most active chemical constituents of OKE-NYR, OKI-KRN and OKI-NYR smoldered volatiles were prepared in proportions in which they occurred in respective blends (Table 4.25).

Table 4.25: Chemical composition of SBAC1, SBAC2 and SBAC3 blends

Blend	Chemical constituents	Composition ratio
SBAC1	Estragole: Eucalyptol: Geraniol: Nerol: Neryl acetate: (*E*)-β-ocimene: α-humulene: (*E*)-β-caryophyllene: (*E*)-β-farnesene	12: 14:8:2:2:5:1:1
SBAC2	Estragole: Eucalyptol: Geraniol: Nerol: Neryl acetate: (*E*)-β-ocimene	12:14:8:2:2:4
SBAC3	Estragole: Eucalyptol: Geraniol: Nerol: Neryl acetate: (*E*)-β-ocimene: Limonene	12:14:8:2:2:4:3

The mean values of bees attracted to the SBAC1, SBAC2 and SBAC3 blends are presented in Figure 4.27.

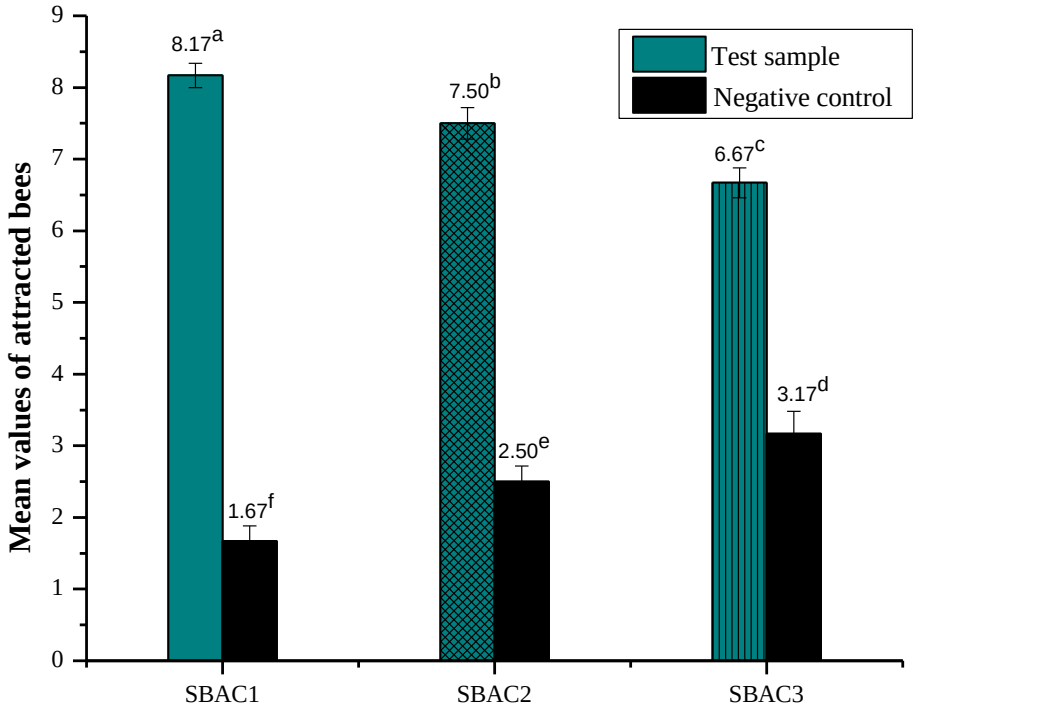


Figure 4.27: Honey bee attractant activity of SBAC1, SBAC2 and SBAC3 synthetic blends

From the results presented in Figure 4.27, honey bees' preferences for synthetic blends of the most attractive *Ocimum* species smoldered volatiles were higher than their preference for negative control. Significant variation in honey bees' preferences for three synthetic blends was observed. SBAC1 blend was significantly more attractive with a mean value of attracted bees of 8.17 ± 0.17 as compared to SBAC2 and SBAC3 blends with respective values of 7.50 ± 0.22 and 6.67 ± 0.21 ($p<0.05$).

4.9.4 Responses of honey bees to serial concentrations of the most active (SBAC1) blend

The SBAC1 blend was diluted by adding acetone to neat blend and assayed against equivalent amounts of diulent (acetone) in Y-tube olfactometer. Minimum Efficative Concentration attracting 50% (MEC_{50}) and 75% (MEC_{75}) of test honey bees were determined by probit analysis as 6.9 and 15.4 µg/µl, respectively (Figure 4.28 and Appendix 7).

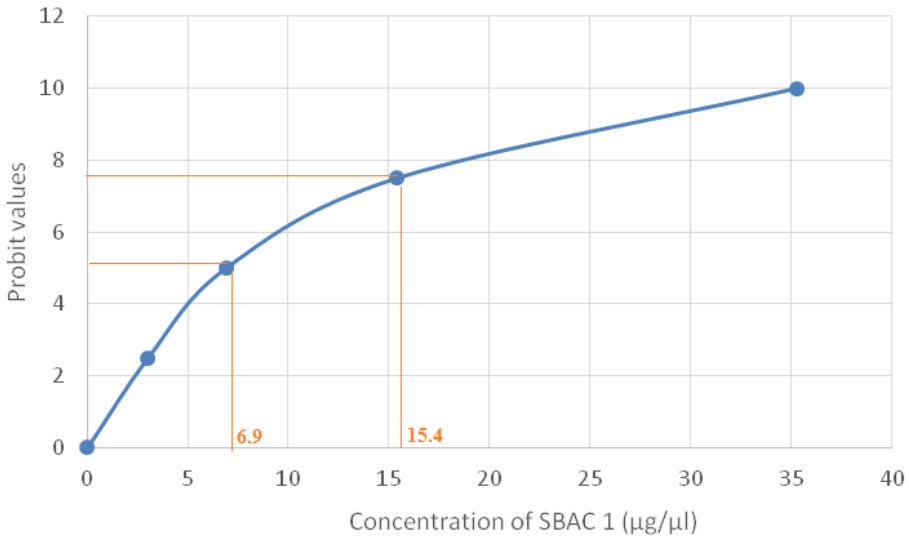


Figure 4.28: A plot of probit values against concentration of the most active synthetic blend (SBAC1)

CHAPTER FIVE

DISCUSSION

5.1 Chemistry of essential oils volatiles of selected *Ocimum* species

Results of this study indicate that respective mean percent essential oil yields of *O. kenyense, O. kilimandscharicum* and *O. lamiifolium* of 0.39%, 0.49% and 0.33% were within the yield range of *Ocimum* species (Table 4.1). Generally essential oil yields of *Ocimum* species range from 0.2 to 1.0% (v/w) but can be as high as 1.7% (v/w) depending on source and developmental stage of plants (Vani, *et al*., 2009).

In this study, chemical composition of essential oils of *O. kenyense, O. kilimandscharicum* and *O. lamiifolium* was determined and remarkable variations based on species and agro ecological zone of origin observed (Table 4.2, 4.3 and 4.4). In general, chemical compositions of studied *Ocimum* species essential oils were found to be comparable to those of similar species' populations growing in various parts of the world (Mwangi *et al*., 1994, Bekele *et al*., 2009 and Runyoro *et al*., 2010).

The essential oil volatiles of both OKE-LKP and OKE-NYR bore a chemical similarity to essential oil of *O. kenyense* species from Ngong area in Kenya. Eucalyptol [**22**] (38.0%) and estragole [**37**] (24%) were reported as major constituents of *O. kenyense* essential oil from Ngong by Mwangi *et al.,* (1994). On the other hand, essential oil of *O. kenyense* from Nairobi area in Kenya contained eucalyptol [**22**] (36.93%), β-selinene [**36**] (23.07%), estragole [**37**] (12.86%) and isoeugenol [**38**] (8.23%) as the major constituents (Bekele *et al.,* 2001). In this work, description of estragole-eucalyptol chemo type of Kenyan *O.*

kenyense is supported by findings of previous studies by Mwangi *et al.*, (1994) and Bekele *et al.,* (2001).

The essential oil of *O. kilimandscharicum* populations from Kirinyaga and Nyeri Counties were chemically different from essential oil of populations of the same species from Siaya County in Kenya due to notable differences in their camphor contents. Camphor [**21**] (70%), eucalyptol [**22**] (7.2%), limonene [**23**] (6.23%) and camphene [**24**] (5.07%) were identified as major constituents of essential oil of *O. kilimandscharicum* from Siaya (Bekele *et al.*, 2009). A study on Nigerian *O. kilimandscharicum* essential oil led to idenfication of methyl eugenol [**25**] (40.4%), borneol [**26**] (11.9%) and linalool [**27**] (10.6%) as its major constituents (Oladipupo *et al*., 2014).

In this work, description of camphor chemo type of Kenyan *O. kilimandscharicum* essential oil is also supported by a previous study by Bekele *et al.,* (2009). Elsewhere, eucalyptol and methyl eugenol chemo types of *O. kilimandscharicum* have been described in Rwanda (Ntezurubanza *et al.*, 1984) and Nigeria (Oladipupo *et al*., 2014), respectively Essential oils of OLA-NYD and OLA-NKU were chemically different from Tanzanian *O. lamiifolium* species' essential oil. Bornyl acetate [**28**] (30.36%), *o*-cymene [**29**] (11.4%) and camphene [**24**] (5.9%) were identified as major compounds of *O. lamiifolium* essential oil from Tanzania (Runyoro *et al*., 2010).

Alpha-Phellandrene content in essential oils of OLA-NYD and OLA-NKU (12.36-13.01%) were comparable to that of *O. lamiifolium* species from Ethiopia. Bornyl acetate and sabinene chemo types of *O. lamiifolium* were previously described in other studies in

Ethiopia (Kifle *et al.,* 2007) and Tanzania (Runyoro *et al.,* 2010), respectively. Ethiopian *O. lamiifolium* essential oils were characterized by β-sabinene [**30**] (31.28%), α-phellandrene [**31**] (13.34%) and 3-octan-1-ol [**32**] (13.42%) (Kifle *et al*., 2007). In this work, description of α-phellandrene chemo type of Kenyan *O. lamiifolium* essential oil is given for the first time.

5.2 Chemistry of fresh and smoldered volatiles of selected *Ocimum* species

Chemical compositions of fresh volatiles of *O. kilimandscharicum, O. kenyense* and *O. lamiifolium* species are being reported for the first time in this study. In general, variations in chemical compositions and percent concentrations of fresh and smoldered volatiles' chemical constituents based on species and agro ecological zone of origin were observed (Table 4.5, 4.6, 4.7, 4.8, 4.9 and 4.10) in this study.

Previous studies on fresh volatiles of O. *basilicum* species from China led to identification of α-bergamotene (57.1%), cedrene (16.4%), eugenol (13.1%) and δ-cadinol (11.8%) as its major chemical constituents (Jiang et al., 2016). In a separate study, GC-MS analysis of head space-solid phase micro extraction (HS-SPME) of volatiles of five different cultivars of *O. basilicum* grown under ecological and conventional cultivation in Czech Republic led to identification of four major constituents. Linalool (16-32%), eucalyptol (3-20%), eugenol (9-22%) and α-bergamotene (1-20%) were identified in all investigated cultivars of *O. basilicum* species from Czech Republic (Klimankova *et al*., 2008).

The findings of this study concur with those of previous studies that have demonstrated variations in volatile chemical constituents of the same *Ocimum* species associated with

edaphic and climatic factors of their respective agro ecological zones of origin (Wossa *et al*., 2008). Solar irradiation (Loughrin and Kasperbauer, 2003), temperature (Chang, 2005), rainfall (Chang *et al*., 2008) and soil type (Pushpangadan and George, 2012) among others, have been reported to contribute significantly to observed variations in quality and quantity of volatile emissions in *Ocimum* species.

There is limited literature on chemical composition of smoldered volatiles of *Ocimum* species. According to Ciccioli *et al.* 2014, smoldering enhances the rate of terpenes emission as well as cause changes in chemical profile of plant's scent due to oxidation and degradation processes. Therefore, occurence of high number of chemical constituents in investigated *Ocimum* species' smoldered volatiles could be attributed to enhanced rate of terpene emission during smoldering process. Smoldered volatiles of *O. kenyense*, *O. kilimandscharicum* and *O. lamiifolium* species were characterized by high terpenoid contents of 31.47-78.52% as compared to fresh volatiles' terpenoid contents of 25.12-66.28%.

5.3 Comparative chemistry of selected *Ocimum* species' volatiles

Variations in chemical compositions of fresh, smoldered and essential oil volatiles of *O. kenyense*, *O. kilimandscharicum* and *O. lamiifolium* species were observed in this study and are described in sections 5.3.1, 5.3.2 and 5.3.3, respectively.

5.3.1 Comparative chemistry of *O. kenyense* volatiles

A total of nineteen, sixteen and twenty-two chemical constituents were identified in essential oil, fresh and smoldered volatiles of *O. kenyense* species from Laikipia (OKE-LKP) and Nyeri (OKE-NYR) (Table 4.2, 4.5 and 4.7). Variations in number of chemical constituents in essential oil, fresh and smoldered volatiles could be attributed to steam distillation and smoldering of *O. kenyense* species plant material. All investigated volatiles of *O. kenyense* species had high benzenoid content of 34.4-43.9%, with exception of OKE-NYR essential oil volatiles whose benzenoid content was only 6.58%. Monoterpenoid and sesquiterpenoid contents of *O. kenyense* species volatiles were reported as 14.1-39.6% and 3.45-27.9%, respectively.

Eucalyptol [**22**] (10.7-27.9%) and estragole [**37**] (18.3-35.1%) were identified as major constituents of OKE-NYR and OKE-LKP essential oil and smoldered volatiles. Interestingly, estragole [**37**] and eucalyptol [**22**] respective content of (1.05-1.15%) and (6.10-8.80%) was reported in fresh volatiles of OKE-LKP and OKE-NYR. Steam distillation and smoldering processes seemed to enhance emission of both eucalyptol and estragole in *O. kenyense* species. *p*-Ethylacetophenone [**68**] (9.65-11.31%) and *p*-methoxyacetophenone [**69**] (8.74-11.68%) dominated fresh volatiles of OKE-LKP and OKE-NYR but were absent from chemical profiles of both smoldered and essential oil volatiles.

β-Pinene [**55**] (13.0-13.3%) was reported as a major constituent of fresh volatiles of *O. kenyense* species from Laikipia (OKE-LKP) and Nyeri (OKE-NYR). On the other hand, β-pinene [**55**] (1.06-4.73%) was identified as a minor constituent of essential oil and

smoldered volatiles of OKE-LKP and OKE-NYR. Therefore, steam distillation and smoldering of *O. kenyense* species' plant material led to decreased emission of β-pinene [**55**] as well as loss of both *p*-ethylacetophenone [**68**] and *p*-methoxyacetophenone [**69**]. Qualitative and quantitative variations in chemical compositions of essential oils, fresh and smoldered volatiles of OKE-LKP and OKE-NYR could be attributed to differences in edaphic and climatic conditions of Nyeri and Laikipia agro-ecological zones (Baker, 1967).

5.3.2 Comparative chemistry of *O. kilimandscharicum* volatiles

A total of twenty-nine, thirty and tweny-nine chemical constituents were identified in essential oil, fresh and smoldered volatiles of *O. kilimandscharicum* species from Kirinyaga (OKI-KRN) and Nyeri (OKI-NYR) (Table 4.3, 4.6 and 4.9). Steam distillation and smoldering of *O. kilimandscharicum* species plant material did not affect the number of chemical constituents reported in its volatiles significantly. Monoterpenoid (49.1-75.1%), sesquiterpenoid (4.2-23.5%) and benzenoid (2.9-25.7%) content was reported in all investigated volatiles of *O. kilimandscharicum* species from both Kirinyaga (OKI-KRN) and Nyeri (OKI-NYR).

Essential oil volatiles of OKI-KRN and OKI-NYR had the highest monoterpenoid content of 72.9-75.1% followed by smoldered and fresh volatiles with respective monoterpenoid contents of 54.0-64.4% and 49.1-56.0%. Camphor [**21**] (12.6-27.4%) was identified as a major chemical constituent of all investigated *O. kilimandscharicum* species' volatiles with exception of *O. kilimandscharicum*-Nyeri (OKI-NYR) species' fresh volatiles. Eucalyptol [**22**] (19.9%) and linalool [**27**] (19.2%) were reported as major constituents of OKI-NYR fresh volatiles while its camphor [**21**] content was reported as 1.42%.

Steam distillation and smoldering of *O. kilimandscharicum* species' plant material seemed to enhance emission of geraniol [**17**]. In *O. kilimandscharicum*-Kirinyaga (OKI-KRN) species, geraniol content of 14.5%, 3.68% and 10.67% was reported in essential oil, fresh and smoldered volatiles, respectively. Similarly, emission of geranial [**16**] and neral [**18**] was enhanced by steam distillation hence the two minor constituents were only reported in OKI-KRN essential oil volatiles. Nerol [**19**] was reported both in essential oil and smoldered volatiles of OKI-KRN.

Qualitative and quantitative variations in chemical compositions of essential oil, fresh and smoldered volatiles of OKI-KRN and OKI-NYR could be attributed to differences in edaphic and climatic conditions of Kirinyaga and Nyeri agro-ecological zones as indicated in Appendix 1 (Baker, 1967). Other studies have shown that temperature of an agro ecological zone influences accumulation of some chemical constituents in *Ocimum* species.

According to Pushpangadan and George, (2012), accumulation of eugenol [**42**] and linalool [**27**] occurred at 25 ºC while accumulation of camphor [**21**] occurred at 15 ºC. Therefore, favourable temperature conditions of 17.4 ºC and 20.6 ºC in Nyeri and Kirinyaga may have contributed to accumulation of camphor [**21**] (17.3-27.6%) in both OKI-KRN and OKI-NYR volatiles. Presence of linalool [**27**] (19.2%) and eugenol [**42**] (3.95%) was only reported in fresh volatiles of OKI-NYR possibly due to relatively low temperatures in Nyeri agro ecological zone that favoured accumulation of the two chemical constituents (Appendix 1).

5.3.3 Comparative chemistry of *O. lamiifolium* volatiles

A total of thirty-two, twenty-nine and fifty chemical constituents were identified in essential oil, fresh and smoldered volatiles of *O. lamiifolium* species from Nyandarua (OLA-NYD) and Nakuru (OLA-NKU) (Table 4.4, 4.7 and 4.10). Steam distillation and smoldering of *O. lamiifolium* species plant material, led to enhanced emission of volatile chemical constituents as evidenced by their high numbers in both essential oil and smoldered volatiles.

Monoterpenoid content of 15.2-36.5% was reported in all investigated volatiles of OLA-NYD and OLA-NKU. Essential oil and smoldered volatiles' sesquiterpenoid content in *O. lamiifolium* species' was found to be 21.3-23.1% and 33.4-37.6%, respectively. On the other hand, sesquiterpenoid content of *O. lamiifolium* species' fresh volatiles' was found to be 2.13-7.52%. Therefore, it is worth noting that steam distillation and smoldering of *O. lamiifolium* species plant material led to enhanced emission of sequiterpenoids. Benzenoid content of *O. lamiifolium* species' fresh volatiles was found to be 17.5-24.8% while respective essential oil and smoldered volatiles' contents were reported as 3.04% and12.3%. Decline in benzenoid content occurred as a result of steam distillation and smoldering of *O. lamiifolium* species' plant material.

α-Phellandrene [**31]** (8.41-18.69%) was identified as a major constituent of all investigated volatiles of OLA-NYD and OLA-NKU. Respective α-phellandrene contents of 12.36-13.01%, 11.92-18.69% and 8.41-12.76% were reported in essential oil, fresh and smoldered volatiles of *O. lamiifolium* species. This is an indication that steam distillation

and smoldering of *O. lamiifolium* species' plant material caused a decline in α-phellandrene [**31**] emission.

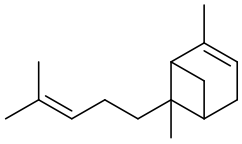
β-Sabinene [**30**] (15.73%) was identified as a major constituent of *O. lamiifolium*-Nakuru (OLA-NKU) fresh volatiles although it was absent in corresponding essential oil and smoldered volatiles. Qualitative and quantitative variations in chemical compositions of essential oils, fresh and smoldered volatiles of OLA-NYD and OLA-NKU could be attributed to differences in edaphic and climatic conditions of Nyandarua and Nakuru agro ecological zones as indicated in appendix 1 (Jaetzold *et al*., 2006; Republic of Kenya: Nakuru County, 2013).

5.4 Comparative chemistry of volatiles of other genus *Ocimum* species

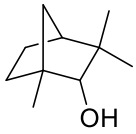
Findings of this study concur with those of related studies which reported remarkable differences in chemical compositions of respective fresh and essential oil volatiles of *O. basilicum* (Tarchoune *et al*., 2013; Yousif *et al*., 1999) and *O. forskolei* (Ali *et al*., 2017; Dekker *et al*., 2011). Fresh volatiles' chemical composition of *O. basilicum* differed from that of its corresponding essential oil where linalool [**27**] (86.4%), geraniol [**17**] (7.48%) and α-bergamotene [**94**] (4.9%) were identified as key components of *O. basilicum* essential oil by Fatope *et al*., (2008).

Chemical profiles of essential oils of *O. forskolei* from Oman and Yemen were different from those of head space volatiles of Eritrean *O. forskolei* (Fatope *et al*., 2008). Analysis of headspace sampled fresh foliar volatiles of Eritrean *O. forskolei* led to identification of (*E*)-β-ocimene [**20**] as a major component whereas estragole [**37**] and linalool [**27**] as well

as endo-fenchol [**95**] and fenchone [**82**] were identified as major components of Omani and Yemeni *O. forskolei* essential oils respectively (Ali *et al.,* 2017; Dekker *et al.,* 2011).



(94)



(95)

Fresh plant volatile emissions comprise of highly volatile constituents which are released into the surrounding air under natural environmental conditions while essential oils comprise of chemical constituents that are released on exposure to steam. Head space sampling gives a more realistic picture of volatile profile emissions detected by insects hence its most preferred for chemo ecological studies (Tholl, 2006). Steam distillation enhances release of less volatile constituents that appear in chemical profiles of essential oils though they may be absent in corresponding fresh volatiles' profiles (Prabhu *et al*., 2009). Extraction of essential oil by steam distillation may lead to loss of some volatile constituents thus explaining their absence in essential oils chemical profiles despite being present in corresponding fresh volatiles' profiles.

5.5 Bee attractant activity of selected *Ocimum* species volatiles in olfactometric bioassay

Selected *Ocimum* species volatiles' attraction to honey bees varied with species, mode of volatile extraction and agro-ecological zone of species' origin. Significant variations in bee attractant activity of essential oil, fresh and smoldered volatiles of selected *Ocimum* species were observed in this study. The individual species within the genus *Ocimum* show

significant variations in the aromatic character, morphological features, chemical compositions and biological activities (Lawrence, 1988; Hiltunen and Holm, 2003).

5.5.1 Bee attractant activity of *O. kenyense* volatiles

Variations in honey bee attractant activity of essential oil, fresh and smoldered volatiles of *O. kenyense* species could largely be attributed to notable differences in classes of compounds in their respective chemical profiles. All investigated volatiles of *O. kenyense* species a had high benzenoid content of 34.4-43.9% with exception of *O. kenyense*-Nyeri (OKE-NYR) essential oils (Table 4.2, 4.5 and 4.8). Therefore, the high bee attractant activity of OKE-NYR smoldered volatiles could be attributed to its monoterpenoid high content of 39.6% in addition to benzenoid content of 34.2%. Smoldered volatiles of OKE-LKP had a low monoterpenoid content of 14.1% in addition to its high benzenoid content of 38.2%, hence the significant varaiation in bee attractant activity of OKE-LKP and OKE-NYR smoldered volatiles (Table 4.8).

5.5.2 Bee attractant activity of *O. kilimandscharicum* volatiles

Honey bee attractant activity of smoldered volatiles of OKI-KRN was significantly high as compared to OKI-NYR. The observed variation in bee attractant activity of smoldered volatiles of OKI-KRN and OKI-NYR could be attributed to notable differences in their monoterpenoid and sesquiterpenoid contents. In OKI-KRN smoldered volatiles, respective monoterpenoid and sesquiterpenoid contents of 64.4% and 14.1% were recorded. On the other hand, respective monoterpenoid and sesquiterpenoid content of 54.0% and 23.5% were recorded in OKI-NYR (Table 4.9). Therefore, the high monoterpenoid content of

OKI-KRN smoldered volatiles could be considered as a key contributor to its high bee attractant activity.

Although essential oil volatiles of *O. kilimandscharicum* species had high monoterpenoid content (72.0-75.08%), their bee attractant activity was lower than that of corresponding fresh and smoldered volatiles. The low activity could be attributed to low sesquitepenoid content of 6.84-7.57% (Table 4.3) reported in *O. kilimandscharicum* species essential oils as compared to fresh and smoldered volatiles' respective sesquiterpenoid contents of 10.2-12.3% (Table 4.6) and 10.9-23.5% (Table 4.9).

5.5.3 Bee attractant activity of *O. lamiifolium* volatiles

Contrary to the observed trend, fresh volatiles of *O. lamiifolium*-Nyandarua (OLA-NYD) species were more attractive to bees as compared to its smoldered volatiles. Fresh volatiles of OLA-NYD had a higher monoterpenoid content of 36.4% (Table 4.7) as compared to its smoldered volatiles content of 19.2% (Table 4.10) hence the most likely reason for its high attractiveness to bees. Interestingly, OLA-NYD and OLA-NKU essential oils were found to be less attractive to honey bees as compared to negative control. *Ocimum lamiifolium* essential oil has a low monoterpenoid content of 21-20-23.23% as compared to respective contents of 27.80-37.73% and 75.08-72.98% in *O. kenyense* and *O. kilimandscharicum* (Table 4.2, 4.3 and 4.4). The variation in monoterpenoid content could be the most probable contributing factor to low bee attractant activity of *O. lamiifolium* essential oils.

5.5.4 Comparative bee attractant activity of selected *Ocimum* species volatiles

Bee attractant activity of essential oil, fresh and smoldered volatiles of selected *Ocimum* species varied significantly with smoldered volatiles being highly preferrable to honey bees (Appendix 4). High bee attractant activity of smoldered volatiles could possibly be attributed to enhanced emission of bee attractant constituents at high temperatures (Ciccioli *et al.,* 2014). Smoldering enhances the rate of monoterpene emission in *Ocimum* species hence the high bee attractant activity observed in smoldered volatiles of investigated *Ocimum* species.

Steam distillation leads to loss of some volatile chemical constituents while at the same time it enhances release of less volatile constituents that are not easily perceived by honey bees (Tholl *et al*., 2006). This could be the most probable factor that contributed to low bee attractant activity of essential oil volatiles of all investigated *Ocimum* species. Exclusion of essential oils of all investigated *Ocimum* species from subsequent olfactometric bioassays was informed by their low bee attractant activity as compared to fresh and smoldered volatiles (Figure 4.19, 4.20 and 4.21).

Smoldered volatiles of all investigated O*cimum* species volatiles were rich in monoterpenoid content while their corresponding fresh volatiles were rich in benzenoid and green leaf volatile chemical constituents. A study on Orchid bee (*Hymenoptera: Apidae*) lures revealed that their attractiveness depended on boiling points of its major chemical constituents such as eucalyptol and eugenol. Eucalyptol (monoterpenoid) is more

volatile than eugenol (benzenoid) hence it could be easily perceived by Orchid bees thus eliciting a stronger response in olfactometric assays (McCravy *et al.,* 2017).

5.5.5 Bee attractant activity of two component blends of *Ocimum* species' volatiles

An antagonistic effect was observed when two component blends of *Ocimum* species' volatiles were tested against negative controls. Bee attractant activity of the fresh volatile blends of two component blends of fresh and smoldered volatiles was significantly lower than activity of individual component species (Table 4.14 and 4.18). This observation could be attributed to emission of bee attractant volatile constituents in proportions and composition other than the ones perceived by honey bees in natural aroma of individual *Ocimum* species could be the most probable cause of the observed decline in bee attractant activity.

Individual blends of fresh and smoldered volatiles comprising of *O. kilimandscharicum* species from both Kirinyaga and Nyeri Counties (KIK-NKF and KIK-NKS) were more attractive to bees as compared to other blends comprising of *O. kenyense* species as one of the components. Great similarity of chemical profiles of *O. kilimandscharicum* species from Kirinyaga and Nyeri could be the most probable reason for the high bee attractant activity of their two component blends (KIK-NKS and KIK-NKF) (Table 4.6 and 4.9).

Blends comprising of fresh and smoldered volatiles of *O. kilimandscharicum* and *O. kenyense* species was less attractive to bees possibly due to variations in classes of chemical constituents present in each species. Monoterpenoids were the most dominant chemical constituents in fresh and smoldered volatiles of *O. kilimandscharicum* species from

Kirinyaga (OKI-KRN) and Nyeri (OKI-NYR). On the other hand, *O. kenyense* species from Nyeri (OKE-NYR)'s most dominant chemical constituents were benzenoids (Table 4.5, 4.6, 4.8 and 4.9). Blending of two attractants has been reported to modify the attraction potential of the total fragrance and often fails to draw species that would visit one of the attractants in pure form (Chadzon and Whitmore, 2002).

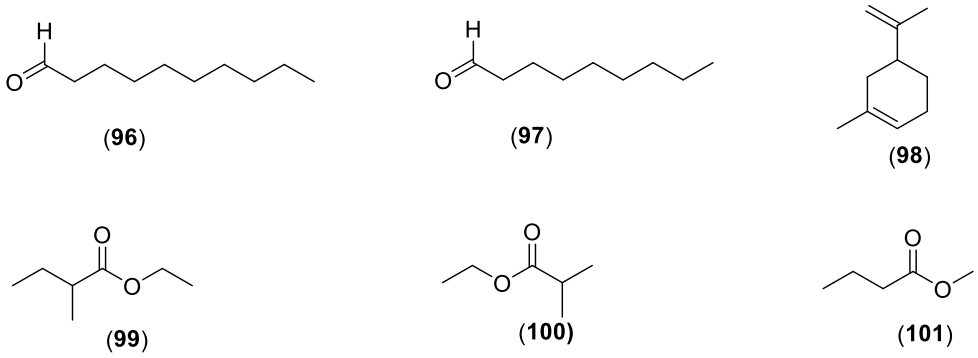
5.5.6 Bee attractant activity of *Ocimum* species volatiles and bee wax

Bee wax is used in modern apiculture as an attractant to lure honey bees into new hives (NAADS, 2007). Honeybees' preference for investigated *Ocimum* species volatiles and their blend with bee wax was lower when bee wax was presented as a positive control as compared to presentation of a negative control in a dual choice olfactometric test (Table 4.15 and 4.17; Figure 4.26). However, an antagonistic effect in bee attractant activities of *Ocimum* species fresh and smoldered volatiles was observed upon addition of bee wax.

Bee attractant activity of all blends of *Ocimum* species and bee wax was lower than that of individual *Ocimum* species volatiles (Table 4.11 and 4.15; Figure 4.20 and 4.12). The antagonistic effect could be attributed to masking effect on honey bees' perception of less volatile but highly attractive constituents of *Ocimum* species volatiles by highly volatile but less attractive constituents in bee wax.

The decline in honey bees' preference for *Ocimum* species volatiles in presence of a positive control could possibly be attributed to presence of highly volatile esters. Bee wax esters have boiling points of 98 ºC to 113 ºC hence they competed for honeybees' attraction with less volatile but attractive *Ocimum* species' volatiles (Bogdanov, 2010). A study by

Kwadha (2017) reported the presence of volatile compounds such as decanal [**96**], nonanal [**97**], sylverstrene [**98**], ethyl-2-methyl butanoate [**99**], ethyl-2-methyl propanoate [**100**] and ethyl propanoate [**101**] in bee wax. In this study, sylverstrene [**98**] (1.38%) was also identified as a minor component in smoldered volatiles of *O. kilimandscharicum*-Nyeri species. On the other hand, ethyl-2-methyl butanoate (ethyl-methylbutyrate) [**99**] (0.91%) was also identified as a minor component of *O. kenyense*-Nyeri essential oil.



Muli and Fraizer (2011) reported traditional use of scented twigs of *Ocimum* plant species to spray molten old combs into hives so as to attract bees in Eastern Kenya. The findings of the present study do not indicate any potentiation effect of blending *Ocimum* species volatiles with bee wax in luring of honey bees into unoccupied hives. Therefore, volatiles of *Ocimum* species found to be highly attractive to bees in olfactometric analysis were deployed in the field without blending with bee wax.

5.6 Bee attractant activity of *Ocimum* species volatiles in field bioassay

The results of field experiment demonstrated that *Ocimum* species' smoldered volatiles had a significantly higher honey bee attractant activity as compared to corresponding fresh volatiles. *Ocimum kilimandscharicum*-Nyeri smoldered volatiles (OK-NYR-SV) treatment was significantly ($p<0.05$) more attractive to bees with a mean occupation time of 30.3

days as compared to bee wax treatments's occupation time of 36.3 days. Bee attractant activity of *Ocimum kilimandscharicum*-Kirinyaga smoldered volatiles (OK-KRN-SV) treatment and bee wax (positive control) were not significantly different with respective mean hive occupation times of 33.6 and 36.3 days. None of the *Ocimum* species' fresh volatiles' treatments lured honey bee colonies into hives regardless of the experimental site and replicate (Figure 4.22 and Appendix 5).

This important observation supports traditional use of smoldered *Ocimum* species plant materials to scent new hives so as to initiate prompt hive colonization. Smoldered volatiles of *O. kilimandscharicum* species from Nyeri and Kirinyaga (OKI-NYR and OKI-KRN) were rich in highly volatile monoterpenoid chemical constituents and hence they could be easily perceived by honey bees. Therefore, smoldered volatiles of *O. kilimandscharium* species elicited a stronger luring response from honey bees scouting for a new nesting site resulting in hive occupations in a field situation (McCravy *et al.*, 2017).

Significantly high preference of honey bee colonies for site 8 (S8) during the field experiment suggests that prompt colonization of a beehive not only depends on attractiveness of applied treatment but also on suitability of placement site (Figure 4.23 and Appendix 5). A suitable hive placement site should be near a source plenty of forage and water, shaded from strong sunlight and wind as well as away from polluted environment such as pesticide sprayed farms (Hilmi *et al.*, 2011). Site 8 (S8) was located in areas of study location that are exposed to moderate sunlight as compared to other sites that were located in areas with dense tree cover.

Hive placement time was also found to be a crucial factor as far as hive colonization is concerned. Highest hive occupation rates of 50% and 75% were recorded in the months of March 2018 and May 2019, whereas no hive occupations were recorded between the month of September 2018 and January 2019. The lowest mean hive occupation time of 25.0 days was recorded in the month of April 2019 indicating an increased tendency of hive occupation (Figure 4.24 and Appendix 5). This observation is a strong indication that for maximum hive colonization to be achieved, reference to bee calendar of a given area is important during placement of baited hives.

Honey bee swarming season in Western Kenya coincides with rainy season of the year during which honey bee colonies migrate due to population increase associated with availability of food. Wambua (2015), reported two honey flow seasons of March to August and October to January in Western Kenya coinciding with long and short rain seasons respectively. However, this study did not report any hive occupation between October 2018 and January 2019 possibly due to depressed rainfall during that season (Figure 4.24). The findings of the present study, strongly concur with Kigatiira *et al*., (1986)'s observation that bee lures should be placed in hives only at those times of the year when migrating colonies usually settle in the area concerned.

5.7 Bee attractant activity of synthetic blends of *Ocimum* species volatiles in olfactometric bioassay

Synthetic blends of *Ocimum* species fresh and smoldered volatiles found to be attractive in both olfactometric and field studies were less attractive to bees as compared to their corresponding natural volatiles in laboratory (Figure 4.19, 4.20, 4.21 and 4.25). Minor

constituents of selected *Ocimum* volatile blends omitted in synthetic blends could play an important part in enhancing bee attractant activity of their respective bee attractant emissions.

Variations in bee attractant activity of synthetic blends of OKE-NYR, OKI-KRN and OKI-NYR fresh and smoldered volatiles can be attributed to remarkable differences in their respective chemical profiles (Table 4.19, 4.20 and 4.21). Synthetic blends of *Ocimum* species smoldered volatiles were generally more attractive to bees as compared to their corresponding fresh volatiles (Figure 4.25). This observation concurs with other results in this study that indicated high bee attractant activity in smoldered volatiles possibly due to high monoterpenoid content (42.40-63.96%) in synthetic blends of smoldered volatiles as compared to corresponding fresh volatiles (32.2-56.5%) (Table 4.19-4.20).

5.7.1 Bee attractant activity of various synthetic blends of *O. kenyense* species

Subtraction bioassay of *O. kenyense* smoldered volatiles' synthetic blend revealed that monoterpenoid and benzenoid compounds were key contributors to bee attractant activity of synthetic blend of OKE-NYR smoldered volatiles (Table 4.22). A significant decrease in mean values of bees attracted to blend A (full blend – [camphor + β-pinene]) from 7.67 ±0.21 to 6.33 ± 0.21 and 6.17 ± 0.17 ($p<0.05$) was observed upon respective subtraction of estragole [**37**] and eucalyptol [**22**].

Subtraction of eucalyptol [**22**] resulted in a higher decline in bee attractant activity of blend A as compared to subtraction of estragole [**37**] (Table 4.22). Eucalyptol [**22**]'s higher bee attractant activity could be attributed to its high concentration of 30.9% as compared to

estragole [**37**]'s concentration of 27.9% in *O. kenyense*-Nyeri (OKE-NYR) smoldered volatiles (Table 4.8). Apart from variation in concentrations, eucalyptol [**22**] is more volatile, with a boiling point of 176 ºC as compared to estragole [**37**]'s boiling point of 216 ºC. In a previous study by McCravy *et al.*, (2017), eucalyptol [**22**], a monoterpenoid compound was reported to be more attractive to Orchid bees as compared to eugenol [**42**], a benzenoid compound due to its high volatility.

Bicyclic monoterpenoid compounds such as camphor [**21**] and β-pinene [**55**] exhibited a low activity against honeybees hence they were excluded from subsequent subtraction bioassays. According to Byer (1992), if removal of a blend component caused either a decline or had no effect on its attractant activity, then the component would be excluded in subsequent subtraction bioassays. Camphor [**21**] and β-pinene [**55**] have been previously shown to be unattractive to honey bees (Huber, 2016) and Euglossine bees (Chadzon and Whitmore, 2002), respectively when assayed in pure form.

Subtraction of sesquiterpenoid compounds such as (E)-β-caryophyllene [**35**] and α-humulene [**51**] from blend A was insignificant to its bee attractant activity possibly due to their low concentrations of 1.5% and 4.4%, respectively and less volatile nature. Therefore, the observed bee attractant activity of smoldered volatiles of *O. kenyense*-Nyeri (OKE-NYR) could largely be attributed to high concentration of eucalyptol [**22**] and estragole [**37**] that constituted 58.8% of the volatiles' content.

5.7.2 Bee attractant activity of various synthetic blends of *O. kilimandscharicum* species

Monoterpenoid and sesquiterpenoid chemical constituents were key contributors to bee attractant activity of synthetic blend of *O. kilimandscharicum*-Kirinyaga (OKI-KRN) smoldered volatiles (Table 4.23). Subtraction of chemical constituents of the two classes led to a significant decline in bee attractant activity of the OKI-KRN synthetic blend. Among the monoterpenoid chemical constituents, geraniol [**17**] and nerol [**19**] were most attractive to bees since their subtraction from blend G (Full blend - [camphor + camphene]) led to a significant ($p<0.05$) decrease in mean values of attracted bees from 7.83 ± 0.17 to 6.16 ± 0.17 and 6.33 ± 0.21, respectively (Table 4.23).

Subtraction of neryl acetate [**83**] and limonene [**23**] led to a significant decrease in respective mean values of attracted bees to blend G from 7.83 ± 0.17 to 6.50 ± 0.22 and 6.67 ± 0.21 ($p<0.05$). Similarly, respective subtraction of sesquiterpenoid chemical constituents such as (*E*)-β-farnesene [**73**] and (*E*)-β-caryophyllene [**35**] also resulted in a significant decline in bee attractant activity of blend G. However, Subtraction of camphor [**21**] and camphene [**24**] from the full synthetic blend did not exhibit a significant effect on its bee attractant activity hence exclusion of the two components from other blends of OKI-KRN smoldered volatiles in subsequent bioassays.

Bee attractant activity of OKI-KRN smoldered volatiles could largely be attributed to the presence of monoterpenoid constituents such as geraniol [**17**], nerol [**19**], limonene [**23**] and neryl acetate [**83**] as well as sesquiterpenoid constituents such as and (*E*)-β-caryophyllene [**35**] and (*E*)-β-farnesene [**73**]. The monoterpenoids and sesquiterpenoids

chemical components constituted 48.3% of the volatiles' content. Apart from high volatility of monoterpenoid chemical constituents of *O. kilimandscharicum* species volatiles, presence of honeybee mimicking constituents could play a key role in its swarm luring activity.

Geraniol [**17**] and nerol [**19**] are key contributors to bee attractant activity of smoldered volatiles of OKI-KRN. The two chemical constituents have been widely described as bee attractants in other studies by Wright *et al*., (2005) and Vareckeen and Doetterl (2010). Interestingly, geraniol [**17**] and nerol [**19**] are also key components of honeybee's Nasonov pheromone (Free and Pickett, 1981). The pheromone elicits clustering activity in a colony during swarming (Janson *et al*., 2005), orients bees to a new nesting site (Abdullah *et al*., 1991), mark nest entrance, orients lost bees and marks rewarding food and water sources (Wells *et al*., 1993).

Bee attractant activity of synthetic blend of smoldered volatiles of *O. kilimandscharicum* species from Nyeri County (OKI-NYR) could largely be attributed to contributions of monoterpenoid chemical constituents such as geraniol [**17**], (*E*)-β-ocimene [**20**] and eucalyptol [**22**]. Subtraction of geraniol [**17**], (*E*)-β-ocimene [**20**] and eucalyptol [**22**] from blend P (full blend – [camphor + fenchone]) resulted in a significant decrease in mean values of attracted bees from 6.83 ± 0.17 to 5.50 ± 0.22, 5.67 ± 0.21 and 5.83 ± 0.17 ($p<0.05$), respectively (Table 4.24).

In addition to the three compounds, other monoterpenoid constituents such as limonene [**23**] as well as sesquiterpenoids such as (*E*)-β-caryophyllene [**35**], α-humulene [**51**] and

(*E*)-β-farnesene [**73**] also contributed significantly ($p<0.05$) to bee attractant activity of OKI-NYR smoldered volatiles. Bee attractant monoterpenoid and sesquiterpenoid constituents of OKI-NYR smoldered volatiles constituted 43.5% of volatiles' content. However, bicyclic monoterpenoid constituents such as camphor [**21**] and fenchone [**82**] constituting blend Q, were found to exhibit insignificant bee attractant activity and hence they were excluded from all subsequent bioassays (Byer, 1992).

(*E*)-β-Ocimene [**20**], a key contributor to bee attractant activity of OKI-NYR smoldered volatiles has been identified as a brood pheromone in honey bees (*Apis mellifera*) (Trhlin and Rajchard, 2011). Young larvae emit a highly volatile pheromone in form of (*E*)-*β*-ocimene [**20**] so as to promote foraging activity among the workers in an attempt to accumulate food reserves (Le Conte *et al*., 2001). The high bee attractant activity exhibited by smoldered volatiles of OKI-NYR in field assay could be attributed to presence of (*E*)-β-ocimene [**20**], which was absent in OKI-KRN volatiles.

Apart from being attractive to honey bees, (*E*)-β-ocimene [**20**] is also highly volatile with a boiling point of 50 ºC as compared to other attractive constituents such as geraniol and nerol with respective boiling points of 230 ºC and 224 ºC (Jiang *et al*., 2016). The high attractiveness and volatility of (*E*)-β-ocimene [**20**] observed in this study, points to its possibility of acting as an ideal bee attractant as far as luring of honey bee colonies to occupy hives during the swarming season is concerned.

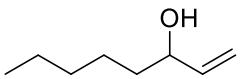
5.7.3 Bee attractant activity of synthetic blends of the most active chemical constituents in olfactometric bioassay

Results of dual choice Y tube olfactometric bioassays of three synthetic blends (SBAC1, SBAC2 and SBAC3) comprising of different combinations of ten chemical constituents led to identification of SBAC1 as the most attractive blend to honey bees (Figure 4.27). The SBAC1 blend constituted of geraniol [**17**], nerol [**19**], neryl acetate [**83**], (*E*)-β-ocimene [**20**], eucalyptol [**22**], estragole [**37**], α-humulene [**51**], (*E*)-β-caryophyllene [**35**] and (*E*)-β-farnesene [**73**]. High bee attractant activity of SBAC1 blend could be possibly attributed to presence of sesquiterpenoids constituents (*E*)-β-caryophyllene [**35**], α-humulene [**51**], and (*E*)-β-farnesene [**73**], which were absent in both SBAC2 and SBAC3 blends.

Presence of limonene **[23**] in SBAC3 blend may have contributed to the observed low bee attractant activity as compared to SBAC1 and SBAC2 blends, in which it was absent. Limonene [**23**] is more volatile (boiling point of 176 ºC) (Gobato *et al*., 2015) hence it diffuses faster through the air as compared to geraniol [**17**], nerol [**19**], estragole [**37**] and neryl acetate [**83**], with boiling points of 216-245 ºC (Jiang *et al*., 2017) hence the observed reduction of activity could possibly be due to spatial repellence.

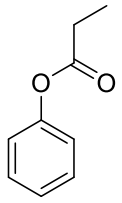
In another study on olfactometric evaluation of spatial repellents for mosquitoes (*Aedes aegypti*) by Kline *et al*., (2003), linalool [**27**] was found to exhibit attractant activity when used alone and spatial repellence activity when used in combination with other attractants such as octen-3-ol [**102**]. Mosquito attractant activity of linalool [**27**] decreased by 50% when it was used in combination with octen-3-ol [**102**] and other attractants. Kvedenok

(1996) reported limonene [**23**] as a bee attractant when used on its own, contrary to the observation on its possible spatial repellence in this study.



(102)

In a previous study, Ladd and Tew (1983), identified eugenol [**42**], anethole [**43**], and phenyl propionate [**103**] as bee attractant compounds. Interestingly, synthetic blend of the three compounds did not attract honey bees even though a blend of eugenol [**42**] and anethole [**43**] was attractive. This observation indicates the need for exclusion of less attractive components during development of swarm lures. Eugenol [**42**] and anethole [**43**] have also been identified as respective chemical constituents of smoldered volatiles of *O. kenyense*-Laikipia (OKE-LKP) and fresh volatiles of *O. kenyense*-Nyeri (OKE-NYR) in this study.



(103)

5.8 Bee attractant activity of pheromonal and non pheromonal chemical constituents

Honey bees respond to *Ocimum* species' bee pheromone mimicking volatile emissions by locating new nesting sites and rewarding food sources as well as increasing foraging activity so as to accumulate food reserves (Le Conte *et al*., 2001). Bee attractant activity of smoldered volatiles of *O. kilimandscharicum* species could be attributed to effect of both

pheromonal and non pheromonal chemical constituents. Monoterpenoids (eucalyptol [**22**], neryl acetate [**83**]) and limonene [**23**]), sesquiterpenoids (α-humulene [**51**], (*E*)-β-caryophyllene [**35**] and (*E*)-β-farnesene [**73**]) and benzenoids (estragole [**37**]) are key non pheromonal constituents of *O. kilimandscharicum* species' volatiles.

Bee attractant activities of non pheromonal chemical constituents such as limonene [**23**], (*E*)-β-caryophyllene [**35**], (*E*)-β-farnesene [**73**] and estragole [**37**] have been described in a review paper by Vareckeen and Doetterl (2010). Prior to this study, there was limited information on attraction of eucalyptol to honey bees although it has been previously described as key component of bumble bee (*Bombus terrestris*) aggregation pheromone (Dornhaus *et al.,* 2003).

Pheromonal chemical constituents such as geraniol [**17**], nerol [**19**] and (*E*)-β-ocimene [**20**] (Free and Pickett, 1981) have been identified as key contributors of bee attractant activity of smoldered volatiles of *O. kilimandscharicum* species in this study. Absence of pheromone constituents such as geraniol [**17**] and nerol [**19**] in *O. kenyense* and *O. lamiifolium* species volatiles explains their lower attraction to bees to as compared to *O. kilimandscharicum* species. Lower attraction of *O. lamiifolium*-Nyandarua (OLA-NYD) fresh volatiles as compared to its corresponding smoldered volatiles contrary to observed trend was due to presence of (*E*)-β-ocimene [**20**], a pheromonal chemical constituent in fresh volatiles of OLA-NYD.

5.9 Contribution of this study to new knowledge

This work presents important findings on comparative chemical composition of essential oil, fresh and smoldered volatiles of *O. kenyense, O. kilimandscharicum* and *O. lamiifolium* species, traditionally used to lure honey bees in Mount Kenya region. Notable variations in chemical compositions of essential oils, fresh and smoldered volatiles of selected *Ocimum* species were reported in this work. For the first time, this study reports chemical compositions of fresh and smoldered volatiles of *O. kenyense, O. kilimandscharicum* and *O. lamiifolium* species as well as existence of α-phellandrene chemotype of Kenyan *O. lamiifolium* species.

Findings of behavioural experiments conducted both in laboratory and field situations led to confirmation of existence of bee attractant activity in essential oil, fresh and smoldered volatiles of the three selected *Ocimum* species. *Ocimum kilimandscharicum* and *O. kenyense* species volatiles are confirmed to have high bee attractant activity as compared to *O. lamiifolium* species volatiles. Smoldered volatiles of *O. kilimandscharicum* from Nyeri (OKI-NYR) and Kirinyaga (OKI-KRN) as well *O. kenyense* from Nyeri (OKE-NYR) were found to be highly attractive to honey bees both in olfactometric and field bioassays.

Subtraction bioassays of synthetic blends of smoldered volatiles of *O. kilimandscharicum*-Kirinyaga (OKI-KRN), *O. kilimandscharicum*-Nyeri (OKI-NYR) and *O. kenyense*-Nyeri (OKE-NYR) led to identification of nine key bee attractant chemical constituents. The identified constituents included geraniol [**17**], nerol [**19**], (*E*)-β-ocimene [**20**], eucalyptol [**22**], estragole [**37**], (*E*)-β-caryophyllene [**35**], α-humulene [**51**], (*E*)-β-farnesene [**73**] and

neryl acetate [**83**]. SBAC1 blend was formulated using the nine constituents and its minimum efficative concentration attracting 50% (MEC_{50}) and 75% (MEC_{75}) of test bees found to be 6.9 and 15.4 µg/µl, respectively.

This study documents for the first time, scientific evidence of bee attractant activity of investigated *Ocimum* species volatiles based on findings of behavioral studies conducted both in laboratory and field situations. Findings of this study have demonstrated that chemo-ecological interactions of honey bees and aromatic plants are very crucial in prompting bee hive colonization. Successful hive colonization has the potential to contribute towards food security, manufacturing and improved livelihoods thus addressing some aspects of Kenya's big four agenda, Vision 2030 and Sustainable Development Goals (SDGs).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

- Major chemical constituents of essential oils were identified as eucalyptol [**22**] (20.2-24.6%) and estragole [**37**] (22.2-35.1%) in *O. kenyense*; camphor [**21**] (21.2-27.4%) in *O. kilimandscharicum* and α-phellandrene [**31**] (12.4-13.0%) in *O. lamiifolium*.
- β-Pinene [**48**] (13.0-13.3%) was identified as a major constituent of *O. kenyense* from both Laikipia and Nyeri while linalool [**27**] (19.2%) and eucalyptol [**22**] (19.9%) were identified as respective major constituents of fresh volatiles of *O. kilimandscharicum* from Nyeri. On the other hand, camphor [**21**] (19.4%) was identified as major constituent of fresh volatiles of *O. kilimandscharicum* from Kirinyaga while β-sabinene [**30**] (15.7%) and α-phellandrene [**31**] (11.9-18.7%) were identified as respective major constituents of fresh volatiles of *O. lamiifolium* from Nakuru and. Nyandarua
- Eucalyptol [**22**] (10-7-27.9%) and estragole [**37**] (18.3-30.9%) were identified as major constituents of smoldered volatiles *O. kenyense* from Laikipia and Nyeri. Camphor [**21**] (12.6-17.3%) was identified as a major constituent of smoldered volatiles of *O. kilimandscharicum* from Kirinyaga and Nyeri while α-phellandrene [**31**] (8.4-12.7%) was identified as a major constituent of smoldered volatiles.*O. lamiifolium* from Nyandarua and Nakuru.
- Smoldered volatiles were found to be more attractive to honey bees as compared to fresh and essential oil volatiles when tested in both laboratory and field situations. Antagonistic effects were observed when *Ocimum* species' fresh and smoldered

volatiles were tested for bee attractant activity in combination with bee wax as well as in combination with one another. *Ocimum kenyense* smoldered volatiles from Nyeri (OKE-NYR), *O. kilimandscharicum* species from Kirinyaga (OKI-KRN) and Nyeri (OKI-NYR) were found to be highly effective in prompting hive colonization as compared to positive control (bee wax).

- Subtraction bioassays of synthetic blends of smoldered volatiles of OKE-NYR, OKI-KRN and OKI-NYR led to identification of a highly attractive nine component SBAC1 blend. The blend consisted of geraniol [**17**], nerol [**19**], (*E*)-β-ocimene [**20**], eucalyptol [**22**], (*E*)-β-caryophyllene [**35**], estragole [**37**], α-humulene [**51**], (*E*)-β-farnesene [**73**] and neryl acetate [**83**].

6.2 Recommendations

- This study has provided scientific evidence supporting use of traditional honey bee luring techniques such as smoking of hive interior parts with *Ocimum* plant species. Therefore, there is need to integrate traditional bee luring technology into modern beekeeping practices in an attempt to address the challenge of low hive colonization.
- Findings of this study have shown that *O. kilimandscharicum* species growing in Mount Kenya region could be cultivated, processed, packed and sold for use as honey bee lures in other parts of the country where they could be unavailable or chemically unsuitable for use as honey bee lures.
- Introduction of *O. kilimandscharicum* species to other parts of the country where they are not indigenous could be done so as to evaluate its effectiveness in initiating hive colonization as compared to the ones from Mount Kenya region.
- Cultivation of *O. kilimandscharicum* species near apiaries could increase the bee density in the area due to the luring scent as well as availability of nectar and pollen since the plant species tends to flower throughout the year.

6.3 Further research

- There is need to conduct field studies to determine the efficacy of synthetic blends of *Ocimum* species smoldered volatiles aroma in luring and establishment of honey bee (*Apis mellifera scutellata*) colonies in both new and old bee hives.
- Studies on bee attractant properties of other *Ocimum* species traditionally used as bee baits in other parts of the country should be carried out with an aim of identifying other highly potent bee attractant chemical constituents.
- Effect of application of crude extracts of selected fresh and smoldered *Ocimum* species on flowering crops with an aim of increasing honey bee visitations, pollination and yields should also be studied and documented.
- Effect of application of *Ocimum* species' volatiles as swarm lures on hive occupation rates and efficiency of bee hive fences used as deterrent to crop raiding elephants should be studied in human-elephant conflict (HEC) hotspots.
- Other genera within Lamiaceae family could also be investigated for their potential to lure honey bees into hives.
- Identified attractants should be exploited in pollination studies to determine their potential in attracting honey bees to crops that are hardly visited by bees.

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APPENDICES

Appendix I: Description of edaphic and climatic features of agro-ecological zones

Sampling site	GPS Coordinates	Soil type	Altitude	Average Temperature	Annual rainfall
Laikipia (Nanyuki)	0˚02'N, 37˚07'E	Luvisolic red brown rocky clay loam soil (Baker, 1967).	1976 metres	16.2 ˚C	819 mm
Nyeri (Kiganjo)	0˚23'S, 36˚56'E	Pale lateritic rusty red soil (Baker, 1967).	2324 metres	17.4˚C	1030 mm
Kirinyaga (Sagana)	0˚47'S, 37˚15'E	Eutric vertisolic black clay soil (Baker, 1967).	2161 metres	20.6 ˚C	1138 mm
Nyandarua (Ol jororok)	0˚03'N, 36˚27'E	Ando luvic Phaeozom dark brown clay loam soil (Jaetzold *et al.*, 2006)	2303 metres	14.0 ˚C	990 mm
Nakuru (Bahati)	0˚28˚N, 36˚06'E	Latosolic red yellow soil (Republic of Kenya: Nakuru County, 2013).	1911 metres	16.4 ˚C	1012 mm

Appendix II: Completely Randomized 8 × 8 Latin Square Block Experimental Design for field bioassay

	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**
C1	1	2	3	4	5	6	7	8
C2	7	8	5	6	3	4	1	2
C3	4	3	2	1	8	7	6	5
C4	6	5	8	7	2	1	4	3
C5	8	7	6	5	4	3	2	1
C6	2	1	4	3	6	5	8	7
C7	5	6	7	8	1	2	3	4
C8	3	4	1	2	7	8	5	6

C=Cluster, S=Site, while 1, 2, 3, 4, 5, 6, 7 and 8 are treatments specifically; **1** (OKKF), **2** (OKENS)**, 3** (OKNF), **4** (OKENF), **5** (PC), **6** (OKKS), **7** (NC) and **8** (OKNS) where; OK-*Ocimum kilimandscharicum*, OKE-*Ocimum kenyense*, F-fresh volatiles, S-smouldered volatiles, PC-positve control and NC-negative control.

	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**
C1	OKKF	OKENS	OKNF	OKENF	PC	OKKS	NC	OKNS
C2	NC	OKNS	PC	OKKS	OKNF	OKENF	OKKF	OKENS
C3	OKENF	OKNF	OKENS	OKKF	OKNS	NC	OKKS	PC
C4	OKKS	PC	OKNS	NC	OKENS	OKKF	OKENF	OKNF
C5	OKNS	NC	OKKS	PC	OKENF	OKNF	OKENS	OKKF
C6	OKENS	OKKF	OKKS	OKNF	OKKS	PC	OKNS	NC
C7	PC	OKKS	NC	OKNS	OKKF	OKENS	OKNF	OKENF
C8	OKNF	OKENF	OKKF	OKENS	NC	OKNS	PC	OKKS

Appendix III: Statistical output showing anova, LSD and means of *Ocimum* species essential oil yield

The SAS System 13:10 Sunday, March 5, 2017

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.76829333	0.06984485	127.96	<.0001
Error	48	0.02620000	0.00054583		
Corrected Total	59	0.79449333			

R-Square	Coeff Var	Root MSE	yield Mean
0.967023	5.763917	0.023363	0.405333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
species	2	0.28432333	0.14216167	260.45	<.0001
location	3	0.28049000	0.09349667	171.29	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
species	2	0.28432333	0.14216167	260.45	<.0001
location	3	0.28049000	0.09349667	171.29	<.0001

The GLM Procedure
t Tests (LSD) for yield

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05`
Error Degrees of Freedom	48
Error Mean Square	0.000546
Critical Value of t	2.01063
Least Significant Difference	0.0149

Means with the same letter are not significantly different.

t Grouping	Mean	N	species
A	0.493500	20	OKI
B	0.397000	20	OKE
C	0.325500	20	OLA

The GLM Procedure
t Tests (LSD) for yield

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	48
Error Mean Square	0.000546
Critical Value of t	2.01063
Least Significant Difference	0.0199
Harmonic Mean of Cell Sizes	11.11111

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	location
A	0.511000	20	NYR
B	0.386000	10	KRN
B			
B	0.373000	10	LKP
B			
B	0.369000	10	NKU
C	0.282000	10	NYD

Appendix IV: Statistical output showing Anova, LSD and means of bees attracted to *Ocimum* species volatiles in olfactometric bioassay

ESSENTIAL OIL VOLATILES OF OKE, OKI AND OLA VERSUS NEGATIVE CONTROL

The SAS System

10:36 Monday, December 4, 2017 54

The GLM Procedure

Dependent Variable: beesattracted

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	41.4861111	3.7714646	3.72	0.0005
Error	60	60.8333333	1.013888		
Corrected Total	71	102.3194444			

R-Square	Coeff Var	Root MSE	beesattracted Mean
0.405457	21.64128	1.006920	4.652778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
species	2	0.19444444	0.09722222	0.10	0.9087
location	3	0.37500000	0.12500000	0.12	0.9460
treatment	6	40.91666667	6.81944444	6.73	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
species	0	0.00000000	.	.	.
location	0	0.00000000	.	.	.
treatment	6	40.91666667	6.81944444	6.73	<.0001

The SAS System

The GLM Procedure

t Tests (LSD) for beesattracted

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	60
Error Mean Square	1.013889
Critical Value of t	2.00030
Least Significant Difference	1.1629

Means with the same letter are not significantly different.

t Grouping			Mean	N	treatment
		A	6.0000	6	EOCOY
		A			
B		A	5.5000	6	EOTOK
B		A			
B		A	5.3333	6	EOCOU
B		A			
B		A	C	5.1667	6	EOTOR
B			C			
B		D	C	4.8333	6	EOTON
B		D	C			
B		D	C	4.8333	6	EOTOL
B		D	C			
B		D	C	4.6667	6	EOCOL
B		D	C			
B	E	D	C	4.5000	6	EOCON
	E	D	C			
	E	D	C	4.1667	6	EOCOR
	E	D				
	E	D	3.8333	6	EOTOU
	E				
	E		3.5000	6	EOCOK
	E				
	E		3.5000	6	EOTOY

Appendix IV: Continued

FRESH VOLATILES OF OKE, OKI AND OLA VERSUS NEGATIVE CONTROL
The SAS System
01:50 Wednesday, December 7, 2017 66
The GLM Procedure

Dependent Variable: beesattracted

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	295.0000000	26.8181818	45.97	<.0001
Error	60	35.0000000	0.5833333		
Corrected Total	71	330.0000000			

R-Square	Coeff Var	Root MSE	beesattracted Mean
0.893939	15.80199	0.763763	4.833333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
species	2	0.0000000	0.0000000	0.00	1.0000
location	3	0.8333333	0.2777778	0.48	0.7000
treatment	6	294.1666667	49.0277778	84.05	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
species	0	0.0000000	.	.	.
location	0	0.0000000	.	.	.
treatment	6	294.1666667	49.0277778	84.05	<.0001

The GLM Procedure
t Tests (LSD) for beesattracted

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	60
Error Mean Square	0.583333
Critical Value of t	2.00030
Least Significant Difference	0.882

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	7.6667	6	FVTOK
A			
B A	7.0000	6	FVTOR
B A			
B A	6.8333	6	FVTOU
B			
B	6.6667	6	FVTON
B			
B	6.5000	6	FVTOL
B			
B	6.1667	6	FVTOY
C	3.3333	6	FVCOL
C			
C	3.1667	6	FVCOU
C			
C	3.1667	6	FVCOY
C			
D C	2.8333	6	FVCON
D C			
D C	2.6667	6	FVCOR
D			
D	2.0000	6	FVCOK

Appendix IV: Continued

SMOLDERED VOLATILES OF OKE, OKI AND OLA VERSUS NEGATIVE CONTROL
The SAS System
11:40 Saturday, December 9, 2017 90
The GLM Procedure

Dependent Variable: beesattracted

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	428.7777778	38.9797980	91.12	<.0001
Error	60	25.6666667	0.4277778		
Corrected Total	71	454.4444444			

R-Square	Coeff Var	Root MSE	beesattracted Mean
0.943521	13.68936	0.654047	4.777778

Source	DF	Type I SS	Mean Square	F Value	Pr > F
species	2	0.8611111	0.4305556	1.01	0.3716
location	3	0.5833333	0.1944444	0.45	0.7151
treatment	6	427.3333333	71.2222222	166.49	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
species	0	0.0000000	.	.	.
location	0	0.0000000	.	.	.
treatment	6	427.3333333	71.2222222	166.49	<.0001

The SAS System
The GLM Procedure
t Tests (LSD) for beesattracted

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	60
Error Mean Square	0.427778
Critical Value of t	2.00030
Least Significant Difference	0.7553

Means with the same letter are not significantly different.

t Grouping		Mean	N	treatment
	A	8.1667	6	SVTOK
	A			
B	A	7.6667	6	SVTON
B				
B	C	7.1667	6	SVTOR
	C			
D	C	6.8333	6	SVTOL
D	C			
D	C	6.8333	6	SVTOU
D				
D		6.3333	6	SVTOY
	E	2.6667	6	SVCOU
	E			
	E	2.6667	6	SVCOY
	E			
	E	2.6667	6	SVCOL
	E			
	E	2.5000	6	SVCOR
	E			
F	E	2.1667	6	SVCON
F				
F		1.6667	6	SVCOK

Appendix IV: Continued

ESSENTIAL OIL, FRESH AND SMOLDERED VOLATILES OF OKE, OKI AND OLA SPECIES

The SAS System
12:30 Saturday, December 9, 2017 90
The GLM Procedure

Dependent Variable: beesattracted

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	692.9351852	69.2935185	72.82	<.0001
Error	205	195.0601852	0.9515131		
Corrected Total	215	887.9953704			

R-Square	Coeff Var	Root MSE	beesattracted Mean
0.780336	20.51591	0.975455	4.754630

Source	DF	Type I SS	Mean Square	F Value	Pr > F
species	2	0.2592593	0.1296296	0.14	0.8727
location	3	0.3750000	0.1250000	0.13	0.9413
treatment	5	692.3009259	138.4601852	145.52	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
species	1	0.0000000	0.0000000	0.00	1.0000
location	3	0.3750000	0.1250000	0.13	0.9413
treatment	5	692.3009259	138.4601852	145.52	<.0001

The GLM Procedure
t Tests (LSD) for beesattracted

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	205
Error Mean Square	0.951513
Critical Value of t	1.97160
Least Significant Difference	0.4533

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	7.1667	36	SVTO
A			
A	6.8056	36	FVTO
B	4.6944	36	EOCO
B			
B	4.6111	36	EOTO
C	2.8611	36	FVCO
D	2.3889	36	SVCO

Appendix V: Statistical output of field bioassay showing anova, LSD and means of hive occupation times

The SAS System
15:47 Thursday, May 30, 2019 759

The GLM Procedure
Class Level Information

Class	Levels	Values
site	8	1 2 3 4 5 6 7 8
cycle	8	1 2 3 4 5 6 7 8
treatment	8	1 2 3 4 5 6 7 8

Number of observations 64

The GLM Procedure
Dependent Variable: hiveoccptime

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	3293.312500	156.824405	2.65	0.0035
Error	42	2483.625000	59.133929		
Corrected Total	63	5776.937500			

R-Square	Coeff Var	Root MSE	hiveoccptime Mean
0.570079	21.83456	7.689859	35.21875

Source	DF	Type I SS	Mean Square	F Value	Pr > F
site	7	617.687500	88.241071	1.49	0.1964
cycle	7	1675.187500	239.312500	4.05	0.0018
treatment	7	1000.437500	142.919643	2.42	0.0357

Source	DF	Type III SS	Mean Square	F Value	Pr > F
site	7	617.687500	88.241071	1.49	0.1964
cycle	7	1675.187500	239.312500	4.05	0.0018
treatment	7	1000.437500	142.919643	2.42	0.0357

The GLM Procedure
t Tests (LSD) for hiveoccptime

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	42
Error Mean Square	59.13393
Critical Value of t	2.01808
Least Significant Difference	7.7594

Means with the same letter are not significantly different.

t Grouping	Mean	N	site
A	39.375	8	7
A			
B A	38.000	8	4
B A			
B A	37.875	8	3
B A			
B A	37.125	8	1
B A			
B A	34.375	8	5
B A			
B A	33.000	8	2
B			
B	31.375	8	6
B			
B	30.625	8	8

Appendix V: Continued

The GLM Procedure
t Tests (LSD) for hiveoccptime

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	42
Error Mean Square	59.13393
Critical Value of t	2.01808
Least Significant Difference	7.7594

Means with the same letter are not significantly different.

t Grouping		Mean	N	cycle
	A	40.000	8	5
	A			
	A	40.000	8	6
	A			
	A	40.000	8	4
	A			
B	A	37.500	8	3
B	A			
B	A	36.500	8	2
B				
B	C	31.375	8	7
B	C			
B	C	31.375	8	8
	C			
	C	25.000	8	1

The GLM Procedure
t Tests (LSD) for hiveoccptime

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	42
Error Mean Square	59.13393
Critical Value of t	2.01808
Least Significant Difference	7.7594

Means with the same letter are not significantly different.

t Grouping			Mean	N	treatment
	A		40.000	8	3
	A				
	A		39.125	8	7
	A				
	A		38.125	8	2
	A				
B	A		38.000	8	4
B	A				
B	A	C	34.875	8	5
B	A	C			
B	A	C	32.250	8	1
B		C			
B		C	30.250	8	6
		C			
		C	29.125	8	8

Appendix VI: Statistical output showing ANOVA, LSD and means of bees attracted to *Ocimum* species volatiles in subtraction olfactometric bioassay

OKE-NYR SYNTHETIC BLEND OF SMOLDERED VOLATILES
The SAS System
16:16 Monday, June 17, 2019
The GLM Procedure

Dependent Variable: beesattracted

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	354.3333333	27.2564103	75.31	<.0001
Error	70	25.3333333	0.3619048		
Corrected Total	83	379.6666667			

R-Square	Coeff Var	Root MSE	beesattracted Mean
0.933275	12.44659	0.601585	4.833333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
syntheticblend	6	0.3333333	0.0555556	0.15	0.9878
treatment	7	354.0000000	50.5714286	139.74	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
syntheticblend	0	0.0000000	.	.	.
treatment	7	354.0000000	50.5714286	139.74	<

The GLM Procedure
t Tests (LSD) for beesattracted

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	0.361905
Critical Value of t	1.99444
Least Significant Difference	0.6927

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	7.6667	6	EUETO
A			
A	7.6667	6	EESTO
A			
A	7.5000	6	SSBTO
B	6.5000	6	BESTO
B			
B	6.3333	6	BCSTO
B			
B	6.1667	6	SESTO
C	4.8333	6	CAPCO
C			
C	4.6667	6	CAPTO
D	3.5000	6	SESCO
D			
D	3.3333	6	BESCO
D			
D	3.1667	6	BCSCO
E	2.1667	6	EUECO
E			
E	2.1667	6	SSBCO
E			
E	2.0000	6	EESCO

Appendix VI: Continued

OKI-NYR SYNTHETIC BLEND OF SMOLDERED VOLATILES

The SAS System

16:50 Monday, June 17, 2019

The GLM Procedure

Dependent Variable: beesattracted

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	343.4166667	14.9311594	35.60	<.0001
Error	120	50.3333333	0.4194444		
Corrected Total	143	393.7500000			

R-Square	Coeff Var	Root MSE	beesattracted Mean
0.872169	13.51608	0.647645	4.791667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
blend	11	0.9166667	0.0833333	0.20	0.9974
treatment	12	342.5000000	28.5416667	68.05	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
blend	0	0.0000000	.	.	.
treatment	12	342.5000000	28.5416667	68.05	<.0001

t Tests (LSD) for beesattracted

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	120
Error Mean Square	0.419444
Critical Value of t	1.97993
Least Significant Difference	0.7403

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	6.8333	6	SEMTO
A			
A	6.6667	6	SSBTO
A			
A	6.6667	6	SMCTO
A			
B A	6.5000	6	SMETO
B A			
B A	6.5000	6	SEYTO
B A			
B A	6.5000	6	SELTO
B A			
B A	6.5000	6	SMHTO
B A			
B A	6.3333	6	SEFTO
B A			
B A C	6.1667	6	SEETO
B C			
B C	5.8333	6	SMOTO
C			
D C	5.5000	6	SEGTO
D			
E D	4.8333	6	CAFCO
E			
E	4.6667	6	CAFTO
E			
E F	4.1667	6	SEGCO
F			
G F	3.5000	6	SMOCO
G F			
G F	3.5000	6	SEECO

Appendix VI: Continued

```
                                    G
                                    G                  3.3333      6    SMECO
                                    G
                                    G                  3.1667      6    SEFCO
                                    G
                                    G                  3.1667      6    SMHCO
                                    G
                                    G                  3.1667      6    SMCCO
                                    G
                                    G                  3.0000      6    SEYCO
                                    G
                                    G                  2.8333      6    SELCO
                                    G
                                    G                  2.8333      6    SEMCO
                                    G
                                    G                  2.8333      6    SSYCO
```

OKI-KRN SYNTHETIC BLEND OF SMOLDERED VOLATILES

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                                The SAS System
                          17:30 Monday, June 17, 2019
                                The GLM Procedure
        Dependent Variable: beesattracted
                                                          Sum of
Source                       DF         Squares     Mean Square    F Value    Pr > F
Model                        19     544.5333333      28.6596491      82.67    <.0001
Error                       100      34.6666667       0.3466667
Corrected Total             119     579.2000000

        R-Square     Coeff Var      Root MSE    beesattracted Mean
        0.940147      12.26633      0.588784              4.800000

Source                       DF       Type I SS     Mean Square    F Value    Pr > F
syntheticblend                9       1.0333333       0.1148148       0.33    0.9627
treatment                    10     543.5000000      54.3500000     156.78    <.0001

Source                       DF     Type III SS     Mean Square    F Value    Pr > F
syntheticblend                0       0.0000000               .          .
treatment                    10     543.5000000      54.3500000     156.78    <.0001

                                   The GLM Procedure
                                  t Tests (LSD) for beesattracted
NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.
                                   Alpha                            0.05
                                   Error Degrees of Freedom          100
                                   Error Mean Square            0.346667
                                   Critical Value of t           1.98397
                                   Least Significant Difference   0.6744
                  Means with the same letter are not significantly different.
                                  t Grouping           Mean      N    treatment

                                       A             7.8333      6    EMSTO
                                       A
                                       A             7.6667      6    SSBTO
                                       A
                                       A             7.5000      6    MTSTO
                                       A
                                  B    A             7.1667      6    MSCTO
                                  B
                                  B    C             6.6667      6    MSFTO
                                  B    C
                                  B    C             6.6667      6    SMLTO
                                  B    C
                                  B    C             6.5000      6    SMATO
                                       C
                                       C             6.3333      6    SMNTO
                                       C
                                       C             6.1667      6    SMGTO
```

Appendix VI: Continued

		D	5.3333	6	CCFCO
		E	4.5000	6	CCFTO
		F	3.5000	6	SMNCO
		F			
	G	F	3.3333	6	SMGCO
	G	F			
	G	F	3.0000	6	SMLCO
	G	F			
	G	F	H 2.8333	6	SMACO
	G	F	H		
	G	F	H 2.8333	6	MSFCO
	G		H		
	G		H 2.6667	6	MSCCO
			H		
		I	H 2.1667	6	MTSCO
		I			
		I	1.8333	6	SSBCO
		I			
		I	1.5000	6	EMSCO

SBAC1, SBAC2 AND SBAC3 SYNTHETIC BLENDS

The SAS System

16:16 Tuesday, June 25, 2019 558

The GLM Procedure

Dependent Variable: beesattracted

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	238.5555556	47.7111111	153.36	<.0001
Error	30	9.3333333	0.3111111		
Corrected Total	35	247.8888889			

R-Square	Coeff Var	Root MSE	beesattracted Mean
0.962349	11.28081	0.557773	4.944444

Source	DF	Type I SS	Mean Square	F Value	Pr > F
syntheticblend	2	0.0555556	0.0277778	0.09	0.9148
treatment	3	238.5000000	79.5000000	255.54	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
syntheticblend	0	0.0000000	.	.	.
treatment	3	238.5000000	79.5000000	255.54	<.0001

The GLM Procedure

t Tests (LSD) for beesattracted

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	30
Error Mean Square	0.311111
Critical Value of t	2.04227
Least Significant Difference	0.6577

Means with the same letter are not significantly different

t Grouping	Mean	N	treatment
A	8.1667	6	SBAC1TO
B	7.5000	6	SBAC2TO
C	6.6667	6	SBAC3TO
D	3.1667	6	SBAC3CO
E	2.5000	6	SBAC2CO
F	1.6667	6	SBAC1CO

Appendix VII: Statistical output showing regression analysis of dose related activity of SBAC1 blend

SUMMARY OUTPUT

Regression Statistics

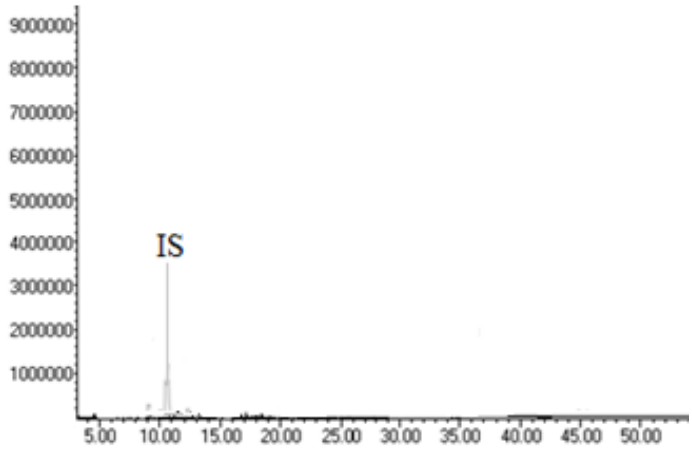
Multiple R	0.9947698
R Square	0.9895669
Adjusted R Square	0.9843504
Standard Error	0.0305148
Observations	4

ANOVA

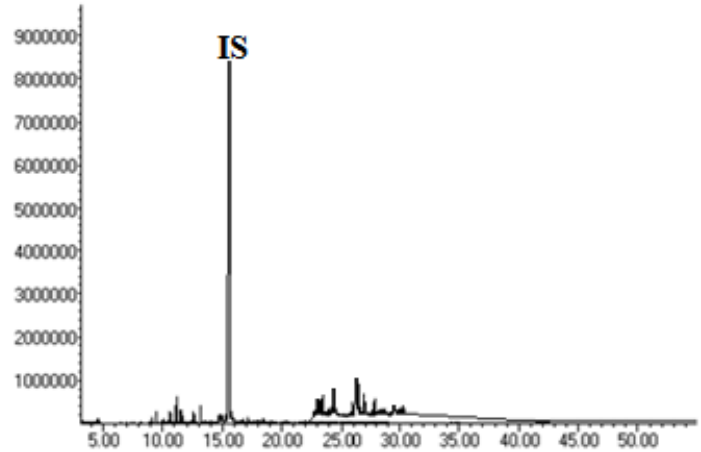
	Df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.176637699	0.17664	189.698322	0.005230207
Residual	2	0.001862301	0.00093		
Total	3	0.1785			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*
Intercept	7.0516353	0.118363747	59.576	0.000281627	6.542357178
X Variable 1	-0.927768	0.067360844	-13.773	0.005230207	-1.21759805

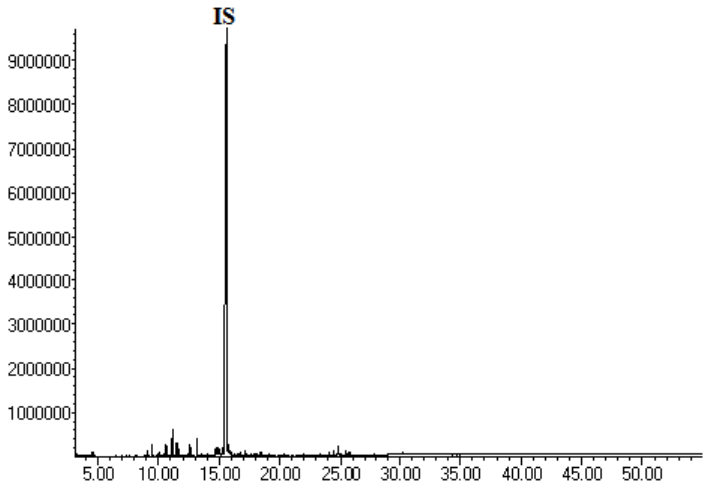
Appendix VIII: Total ion chromatograms of blank and control volatile samples



(A) A total ion chromatogram of an empty glass container (fresh volatiles control)



(B) A total ion chromatogram of glowing charcoal (smoldered volatiles control)



(C) A total ion chromatogram of a blank volatile collection trap (VCT)

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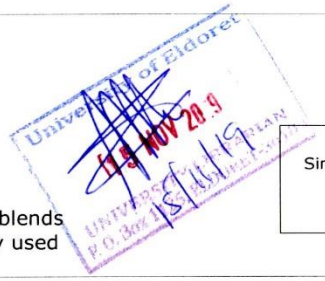

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